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L I S T E R I O S I S

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A. The microorganism of listeriosis.

I. Nomenclature and systematic classification.

The history of listeriosis[173] as an etiologic entity for an infectious disease, showing very different symptoms in animals and humans, properly begins with the accurate description of the microorganism by Murray, Webb, and Swann in the year 1926. Meanwhile (1924) during an epidemic among caged guinea pigs and rabbits at Cambridge, a variety of bacteria was isolated with which rabbits could be infected experimentally, and that, during the course of the disease it caused in the experimental animals, resulted in characteristic changes in the leucocyte count with a manifest monocytosis. On the basis of these characteristics, this microorganism, of which the original culture exists today after many passages, acquired the designation Bacterium monocytogenes.

One year later Pirie--as he had already proved--wrote that he had isolated the same strain in South Africa from Tatera lobengulae, a desert jumping rodent, under the name of Listerella hepatolytica, since, with this strain, he was able to cause typical liver changes in infected research animals. After confirming its identity, over the passage of a decade it was agreed that the preferred designation was Listerella monocytogenes, which has since been printed in many textbooks.

In 1939[120] however, it was learned that the generic name Listerella had already been allotted to a Mycetozoa in 1906 by Jahn and to a Foraminifera in 1933 by Cushman.

For this reason Pirie in 1940 proposed the name Listeria monocytogenes. Although most bacteriologists and cataloguers are in agreement with this designation, objections have appeared.

Specifically, in botany there is a kind of orchid named Listera, and in zoologic nomenclature (1939) it appears that Listeria has been, since 1863, the name of a Diptera (Robineau--Desvoidy). Moreover, the same classification has appeared since 1790 under the designation Listeria Necker in the botanical catalogue.

But it happened that meanwhile the designation Listeria monocytogenes had become generally entrenched in Bacteriology, so the International Commission on Nomenclature agreed that this name be reserved as a Genus conservandum (reserved genus) for the Schizomycetes. At the same time the Commission on Botanical Nomenclature was asked to place the name Listeria Necker on the list of Nomina rejicienda (rejected names)[59].

There is hardly any doubt that the strain found in 1924 by Murray, Webb, and Schwann was isolated previously and reported on in connection with established pathologic findings. In

Bergeys Manual of Determinative Bacteriology, for example, a reference is found to the 1911 discovery of Bacterium hepatis by Hülphers in Sweden.

A few writers[16,179,181] however, reporting of late on the discovery of the Swedish scientist, raise contradictions, however, by reference to certain clear discrepancies in the original writings[317]. This will never be clarified, because the original strain of B. hepatis (Hülphers) is unobtainable.

It appears, too, that Listeria was isolated even earlier elsewhere. This supposition can however, be verified in only one study, that was made at the end of the first World War in France. The culture grown by Dumont and Cotoni that was maintained in the Pasteur Institute was identified as L. monocytogenes 20 years later by Paterson. The strain written about by Fraenkel in Germany also closely resembled Listeria. Since it can no longer be obtained, a definite statement on its systematic evaluation is not possible. Evidently this same microorganism has been observed repeatedly in human illnesses and in fatal cases[3,9,103,198,229,294, et al] but not precisely differentiated bacteriologically. -- Some reports in the Anglo-American literature may be evaluated in the same way:[10,12,54,138a]. Perhaps the erysipelas bacteria referred to by Schipp or Broll in 1910/11 was a strain similar to Listeria.

Despite the reports (172, 194) referred to and their inclusion in standard reference works on Bacteriology, the microorganism is still often newly reported.

Myfeldt in 1929 reported the discovery of Bacterium monocytogenes hominis that he obtained in pure culture from the blood of a typical case of listeriosis in Blegdam Hospital, Copenhagen. The supposition that we are here concerned with a variant of Listeria with cultural and biochemical peculiarities cannot be upheld, so that the designation Listeria hominis used later is also to be challenged. The Corynebacterium parvulum described in 1934 by Schultz, Terry, Brice, and Gebhardt, may also be identified as L. monocytogenes. No others class it with the Corynebacterium infantisepticum (Potel, 1951) and included later in Listeria infantiseptica (157c,242d,242i,217,205).

However small specific designations may be made according to the pathologic anatomic or clinical viewpoint for forming subdivisions of a genus, may be tested with specific criteria, so it was decided that designations as L. ovis, L. cunicula, L. bovina, L. gerbilli, L. gallinarym, and so forth, are untenable. Basic findings support the hypothesis that Listeria strains are related to the group of Acidobacteria (150,159). The numbers of conflicts in nomenclature became still larger when the reknowned English

authors Topley, Wilson and Miles viewed the listerias, because of structural differences, not as distinct genera, but, because of morphological and ecological similarities, class them as subgroups of the erysipelas bacteria and call them Erysipelothrix monocytogenes. This kind of naming is erroneous, since it is known that despite many similarities there are such fundamental differences between Listeria and erysipelas bacteria, that a division into two genera--Erysipelothrix and Listeria--is totally justifiable (98,99,122,172,179, 204,242,256a,281b,317).

In the present state of our knowledge, therefore, the designation Listeria monocytogenes Pirie is alone appropriate, and will be used invariably in the following. This statement does not preclude the possibility that, perhaps, still other members of the genus Listeria may be found that are not identifiable with the species L. monocytogenes.

The systematic relationships may be illustrated by the following outline (after Bergey's Manual (38)):

Class: Schizomycetes Naegeli (1857)
1. Order: Eubacteriales Buchanan
Suborder: Eubacterineae (Breed, et al)
8. Family: Corynebacteriaceae (Lehmann and Neumann)
Genus: Listeria (Pirie)
Species Listeria monocytogenes Pirie

While Erysipelothrix and Listeria here appear as genera of the same family, Prevot (208) places these strains in general of different families:

Schizomycetes	
Eubacteriales	Mycobacteriales
Class I. Asporurales (Prevot, 1948)	Class I. Actinomycetales (Buchanan)
Order II. Bacteriales (Prevot 1940)	Order I. Actinobacteriales (Prevot)
Family V. Bacteriaceae Cohn	Family Actinomycetaceae (Buchanan)
Genus: Listeria	Genus: Erysipelothrix

2. Biochemical and Cultural Characteristics.

Disregarding a few minor differences in fermentation characteristics that will later be discussed separately, all of the many studies found to date from all parts of the world show results that are in general agreement, proving that strains of L. monocytogenes should be considered a morphologic, cultural, and biochemical entity. Cultures obtained from animals and men show no tinctorial and fermentative differences, and likewise no possibilities of differentiation exist on a cultural-and biochemical basis for strains which have been taken from cases of listeriosis in which the course of the disease, clinically, varied greatly.

Recognition of the Listeria on the basis of their cultural and biochemical characteristics is not very practical and the usual method is, since there are manifold possibilities of confusion, which will be discussed fully in the second on differential diagnosis (p. 115ff).

Meanwhile the following are the most important characteristics, that must be unequivocally observed when testing suspected strains. (Techniques[161]).

Morphology: L. monocytogenes is a gram-positive short rod that forms no spores. Capsules have, up to now, never been demonstrated. In young cultures predominantly coccoid bacteria forms that are 0.5 microns wide and 1-2 microns long are found that appear frequently to have somewhat pointed ends, and some in pairs lay against or behind each other or are layered in rings of from 3 to 8 rows. Generally the stratification is not typical; among V-forms, diploid forms, and organisms arranged in parallel are interspersed thin, slightly bent organisms 2 to 5 microns in length. Clubshaped forms or pleomorphic degenerative forms have never been observed.

In very young cultures about 3 days old predominantly rodlike forms are found, which are generally not seen unless one specifically searches for them.(Figure 2). In cultures a few days old rough structures may be isolated and are readily detectable because the culture changes almost entirely into the rough form (Figure 3). These rough forms may be 6 to 20 microns, in lengh but, exceptionally, may be up to 275 microns in length.[15,99,172,179f,204,242 et al)]

The tinctorial behavior is not unique. Young cultures are, independent of the substrate and the cultural composition, without

exception, gram-positive. After 2 to 5 days of incubation gram-lability definitely appears, and an increasing number of the bacteria lose the Gram strain upon differentiation with acetone-alcohol or 96 percent alcohol(172), a characteristic that is also found in other Corynebacteria, e.g., the microorganism of diphtheria.

If the duration of the decoloration is extended to 3-5 minutes about half of all the bacteria in such young cultures will react gram-negatively, if they are being cultured on blood-agar. Cultivation on glucose-containing medium promotes gram-positivity, so that after 72 hours incubation all the bacteria are always gram-positive still, even though the process of decoloration is extended to 10 minutes(242i). The threadlike forms are distinguished by their greater Gram-fastness; only in cultures many weeks old does one find a partial decoloration(179).

Figure I. L. monocytogenes; Morphology of a 24 hour old culture on blood-agar; Gram staining; 800 magnification.

Figures 2 and 3. L. monocytogenes; Little rods and threadlike forms from rough cultures after 48 hours on blood agar; Gram staining; magnified 1000 times.

Small polar bodies after Ernst-Babes are never detected in Listeria; nevertheless, in differentiation by the Gram staining the middle part of the bacterium decolorizes quicker than the ends thus the impression of

the presence of polar bodies may be formed[172]. Listeria never show bands or zones and are also not acid-fast. The individual dyes that are commonly used in bacteriology and histology are not suitable for staining them by the usual methods. For example, the use of methylene blue has been frowned upon for a long time[179f]. The most dependable, particularly for histologic purposes, is Gram staining; Giemsa and Leishman stain techniques may also be recommended.

In cross sections the detection may be accomplished well with the aid of the V. Jensen-modification of Claudius' stain. Also the exclusive use of hematoxylin without eosin contrast staining, as is, for example, used in the socalled red elastica (tunica elastica?) staining, yields good pictures in histologic preparations.

Flagellation: L. monocytogenes is flagellated. However the detection of the presence of flagella by staining is in Listeria connected with considerable difficulty, as a result of which contrary reports are to be found on precisely this point. Many competent authorities have ascertained the presence of motility but do not account for flagellae. A few authors[40, 82,115,240, et al] find monotrichous, polar, flagellation; on the contrary, Paterson found four flagellae arranged peritrichically. This difference was later explained (88) in that at lower incubation temperatures the formation of peritrichically arranged flagellae resulted, while at 37°C. a

reversible flagella--inhibition ensued, that led either to total immobility or to the development of only a polar flagella. Nyfeldt, by one of the methods described by Morton (1890) has confirmed the finding of a predominantly peritrichic flagellation of L. monocytogenes but also stated about them that the flagella were easily broken off, after which, usually, only a polar flagella remained.

In order to answer the question thus arisen, it was next attempted to use the electron microscope. The studies carried out to date by Seidel(329) at the Institute for Biology and Medicine, Pharmacologic Division, Berlin-Buch (Dr. R. Jung) have yielded as yet no conclusive results. The Listeria always assimilated flagellae after incubation at 37°C. which led to a monotrichic flagellation at this temperature (Figure 4).

Motility. At hardly any point do the findings differ so greatly as in reports on the motility of Listeria. Without reviewing the numerous reports again here, we can say in summary, that all of the strains of Listeria monocytogenes found to date are motile and are thereby differentiated basically from almost all varieties of Corynebacteria and Erysipelothrix.

The motility is greatest at room temperature. At 30° C. and even at 37°C. it is not fully abolished, since in "stabs" practically all bacteria from young cultures on bouillon show manifestations of

truly active position changes. In older cultures only a strong molecular motion is generally found, but upon longer observation, there always come into the field of vision a few bacteria that are actively moving.

Figure 4. Polar flagellation of L. monocytogenes at 37°C. Electromicrophotograph, taken at the Institute for Medicine and Biology, Pharmacologic Division, Prof. Dr. Jung, Chief, Berlin-Buch. Source of the materials used: the Institute of Nutritional Hygiene of the faculty of veterinary medicine of the Humboldt University in Berlin, Dr. Seidel, oblique chrome lighting. Magnified 18,000 times.

The manner of progression is quite characteristic. Usually it begins with a peacefully lying bacteria by rolling and peculiar swinging movements which give rise to swift, eccentric revolutions, until the bacteria suddenly in one motion coils itself up. Tumbling and rotating motions that can also again change into a state of rest, are a good indication of the presence of Listeria. In vigorously mobile cultures all the bacteria are in swift, directed motions, resembling that of Salmonella. Threadlike forms make a coil-like forward motion, like that known for bacilli. The most elaborate method for the detection of motility consists of the use of 0.2 to 0.4 percent nutrient agar in U-tubes after the method of Vahlne. The bacteria sown in one arm of the tube swarm, at 22 and 37°C., during the course of 1 or 2 days, through the "possible mobility agar" (242f) and appear on the surface of the other arm. Thereby the previously

clear medium becomes progressively more cloudy with the spread of the culture. This progress may be followed easily with the naked eye. About 0.5 cm. under the surface of both arms of the U-tube a stripe is formed, that is formed from a layer of stronger concentration of bacterial growth. In this zone of a diminished aerobiosis the bacterial cultures show better development than under aerobic or strictly anaerobic conditions (see next section). (Figure 5).

Also in agar-gelatin stabs (Figure 6) the bacteria often show, after incubation at room temperature, fuzzy outgrowth zones, which originate because of the motility of the Listeria (240), on solid media after prolonged incubation there results a similar Medusa's-head-like spreading out of the colonies into the immediately surrounding areas of the culture, that should not be confused with the growth of rough colonies.

Relation of growth to oxygen tension. As previously explained (see Fig. 5) Listeria prosper best in a mixture of gases in which the normal oxygen tension is diminished. When oxygen is eliminated and carbon dioxide substituted for it, after the method of Fortner, a luxurious growth results, the same as when the air is fully expelled and coal gas is introduced (188a). Under strictly anaerobic surroundings without substitution of CO_2, growth is poor or is nearly inhibited (2421,281b).

Many freshly isolated strains and all laboratory cultures grow uninhibitedly under the usual aerobic culture methods. But whether this is suitable for the culture of fresh strains also cannot yet be decided, because it appears that in many cases the first passage in diminished oxygen tension or lower redox potential, for example, in sodium thioglycollate bouillon, is easier to accomplish than under aerobic conditions (298). In any case it is established that the Listeria are facultatively aerobically growing bacteria, and that they are microaerophil, or better, carbondioxidophil.

Figure 5. Typical growth of L. monocytogenes in semifluid nutrient agar (after 3 days at 22°C.)

Figure 6. Growth of L. monocytogenes in dextrose-gelatin stabs (after 10 days of incubation at 22°C.)

Relationship to Temperature. The optimal temperature for growth lies between 20 and 37°C. with the greatest proliferative ability between 30 and 37°C. Growth is retarded at room temperature, but attained the same dimensions in 3-4 days as it did after 1-2 days when the temperature was regulated by a thermostat. Temperatures about 20°C. are clearly better for the development of the bacteria than is body temperature. The motility is definitely increased, but diminishes considerably after the culture has been standing for many more days.

Occasionally the growth at 37°C. from natural (organic?) material for research is definitely diminished, with the result that macroscopically visible growth is first seen after 3-5 days of incubation. Important--above all else for practical diagnosis, (see page. 113)--is the rate of growth at relatively low temperatures as determined by Gray and coworkers(87c). Naturally the rate of growth is thereby considerably slower; but after 10-14 days in a refrigerator at 4°C. there is visible growth of the colonies or definite clouding of liquid substrates. -- Cooling to very low temperatures--for example to-20 to -75°C.--that are unavoidable during freeze-drying, will cause no damage, if the freezing process is carried out quickly and thawing out is prevented. The bacteria may be kept in an excellent state of preservation when thus freeze dried (or lyophilized.). The upper limit of growth is from 42 to 44°C. At such temperature there is still sparse growth. According to Murray, Webb, and Swann the bacteria may be killed by remaining at 47-48°C. for 1 1/2 to 6 hours. Other researchers(15,179f) set these limits higher and report survival of bouillon cultures after 1-2 hours at 56°C. but no such survival after heating for a half hour at 60°C. Thick cultures will survive from 15 seconds to 5 minutes (for a particularly heat-resistant strain) at 100°C. according to Zink and coworkers. In studies of bacterial survival, a temperature of 80°C. for 15 seconds sufficed to kill off Listeria in milk(2421). That yields the important,

practical conclusion, that by the prescribed pasteurization it is also assured that the Listeria will be rendered harmless.

Nutritional requirements. The Listeria do not belong to the very fastidious kinds of bacteria. They grow on a simple casein or hydrolyzed gelatin medium with dextrose and a few inorganic salts, if lactoflavin, hemin, and biotin are added (117). In our own extensive research it was shown that the half-synthetic medium of Ivanovics allowed sufficient possibility for growth, and that this was not increased by the addition of para-aminobenzoic acid and vitamin B. concentrates. The contents of a common meat broth-peptone, - bouillon consist, in contrast to simple peptone-water, already of all the substances necessary for growth. Of course, the growth is then only sparse, it becomes luxurious only upon the addition of fermentable carbohydrates in an 0.5--1 percent concentration. Blood or serum are not absolutely necessary but aid in making the growth visible, if it is raised on a deficient substrate.

Relation to pH: Unlimited growth occurs in neutral and weakly alkaline media also in weakly acid media. Greater degrees of acidity are inhibitive; and in media with a pH of less than 5.6-- for example, in dextrose bouillon, that has been inoculated with Listeria and incubated 2-3 days - it often happens that the culture dies, so that inoculation on acidified sugar media are no longer recommended, ordinarily. The bacteria are relatively insensitive to

alkalis. In media with a pH of 9.6 there is still growth (2421,293).

Growth in fluid substrates. In ordinary meat-broth-peptone-bouillon growth is somewhat slow and odorless; only after 1-2 days at 37°C. is there a definite turbidity.

There is likewise a demonstrable relationship between the number of bacteria sowed or inoculated and culture growth. With large inocula growth never fails, with smaller inocula it is usually delayed a few days; when only a few colonies or even only a few individual bacteria are inoculated, the culture generally does not begin to grow (179f).

This condition is perhaps one of the reasons why the culturing from pathologic material is found difficult, while after previous concentration, for example after the research material has been stored for many months in a refrigerator at 4°C. (87c) --the cultures are easily induced to grow.

After many days the bacteria congregate in the cup of the tubes and form there a slimy sediment that can be shaken up only with difficulty. It may be spun up in the form of a characteristic spiral. Besides, there is the picture when the substrate contains a cleavable sugar (0.5-1 percent). In about 18-24 hours relatively small inocula give rise to a very cloudy turbidity, also, that remains in the case of smooth strains for from 1 to several days, until the bacteria

then also forms flocculent sedimentation. Rough strains grow in liquid media with flocculation, and with a thin skin, and form already after 24 hours a granular, friable precipitate. As under laboratory conditions there are many developing intermediate stages between pure smooth and rough forms, there is also in fluid media a transitional stage, in which the beginning turbidity swiftly disappears, as most of the bacteria sink down to the bottom. They can indeed be shaken up, but do not remain in suspension.

Growth of Surface Cultures. On surface cultures the growth is often only sparse, but never rich, even when blood, serum or fermentable sugars are contained by the medium.

On nutrient agar they develop as very small, transparent, dewdroplike, colonies of 0.2-0.4 mm. diameter with a uniformly iridescent appearance.

The colonies become considerably larger on dextrose agar. They attain, after 3-4 days incubation, a diameter of 1-3 mm. and are a grayish white color. A distinctive odor escapes from the colonies that reminds one of sour milk or buttermilk.

On sheep blood agar the colonies are at first very small and on the first day can hardly be seen with the naked eye. After 48 hours at 37°C. they attain a diameter of 0.2-1.5 mm. Inocula from laboratory cultures become somewhat larger, so that after

two days isolated colonies 2 mm. in diameter may be found, if good nutrients are at their disposal. The colonies at first are like dewdrops and are transparent, however, they later become grayish white and opaque. They are surrounded by a narrow but clearly visible beta-hemolytic ring, With weaker hemolysis this is present only under the colonies. It will become visible only after stripping off the growth. Further see the section on hemolysis. Colony formation and the process of dissociation. On surface cultures different kinds of growth and colony forms may be differentiated with most strains. Freshly isolated strains are composed almost exclusively of round, smooth colonies, which after 3 days appear opaque and somewhat whitened with a surface that shines like a mirror (Figure 7). They have a butter-soft consistency and may easily be dispersed in a solution of sodium chloride.

Isolated colonies occasionally become up to 6 mm. in size in blood agar, if the plates are allowed to stand at room temperature and prevented from drying out. After some days the surface that was smooth in the beginning changes; first a swollen border zone is formed, then the center sinks somewhat and a central button becomes visible. These grow as typically seen smooth forms of colonies, that on blood agar are strongly hemolyzed.

Among these S-forms are found in various proportions some weaker hemolyzing colonies, that in contrast to the opaque-white-coloring after 2-3 days appear more transparent and grayish white. Laboratory strains many times show only this form of growth, which was first found because of a somewhat larger diameter of its colonies and a definite, distinguishing surface marking. The border is no longer smooth but somewhat wavy; the button formation and the central depression are more easily distinguishable. A definite radial striation of the border zone is characteristic (Figure 8).

This form of growth may be conceived of as culture variant of the pure smooth form. It tends in varying degrees towards the ability to agglutinate spontaneouly, but is in its consistency usually as soft as butter and behaves in agglutination and absorption tests as a smooth form. To be sure, there are indispensable technical tricks (see page 19) for overcoming the tendency to autoagglutination. The growth of this variant in bouillon results

Figure 7. Smooth colony of L. monocytogenes on blood agar after 48 hours incubation at 37°C. Diameter 1.2 mm.

Figure 8. Culture variation of L. monocytogenes on blood agar after 48 hours at 37°C. Diameter 2.1 mm.

in a uniform turbidity. After many days the bacteria precipitate to the bottom of the tube. A reversal of this form of growth, that may correspond to the I-form of Barber, into the pure smooth form has not been observed, but on the contrary it repeats the dissociation of the smooth form into S- and I- colonies (2421).

This variant is not identical with the rough form. The latter arise from most strains after a longer period of incubation under the influence of the artificial, laboratory environment, and show themselves by only limited hemolysis, and the colony picture that is typical of the rough form (Figure 9). Rough colonies have a brittle, hard consistency, may be dispersed only with difficulty, and generally show a strong spontaneous agglutination. Inoculations in bouillon grow in films and with quicker formation of a granulated sediment. Listeria show in connection with their form of growth two processes of cleavage; one with the change of the smooth, opaque type of culture into a hemolytically less active, culture variant that appears to be more transparent with the first indication of roughness, but still maintaining serologic specificity and finally into the development of typical, rough forms with all the characteristics of the same. The process of dissociation never reverses itself according to our results; generally, it is not easy to find purely rough forms. Relatively oftener mixed forms are found which indeed

appear rough in cultures, but after sowing may be seen to be in fact mixed with smooth forms. Failure to observe these conditions has led to conflicting reports.

Besides these predominanting forms there are a number of culture variants, that fall under the classification as intermediary forms, and transitional stages. One of these may be seen in figure 10. It results from the outgrowth of a rough division from a smooth colony; (so called bomb-form).

Additional types of colonies: Gray and coworkers (871) were able to detect the presence of other, until then unknown, forms of colonies by examining the surface growth on media to which the redox indicator 2,3,5-triphenyl tetrazolium chloride (TTC) had been added. Of nine colony types differing in growth, consistency, surface characteristics, and color, six could be cultivated unchanged. The color showed all variations from cream to bright red, which resulted from variable reduction potentials.

Growth in Stab Cultures. In nutrient or dextrose agar stabs even growth occurs along the length of the channal made by the stab, as long as the concentration of agar is 1.5 percent or higher. On

diminution of the agar content outbursts into the substrate result because of bacterial motility (see page 6). This may also be observed in broth-peptone-gelatine or in dextrose-gelatine media. Often brushlike proliferations appear; or colored excrescences, which may be confused with Erysipelothrix. While this brushlike growth in erysipelas bacteria but also in simplex nutrient gelatine (without dextrose) never fails, in the case of Listeria it is missed, as in this medium at room temperature it appears only as a fine streak without outgrowth, even if the observation is extended over a period of weeks. -- In nutrient agar stabs the culture remains viable for many years.

Figure 9. Rough colony of L. monocytogenes on blood agar after 48 hours at 37°C. Diameter 3 mm.

Figure 10. Splitting up of L. monocytogenes. Bomblike form in the middle of the picture. Enlarged 8 times.

Hemolysis -- As already explained, all Listeria strains may be characterized by their capacity to hemolyze erythrocytes. At least, that is true for all cultures of material that is freshly isolated from pathologic material. This capacity is exerted by them without differentiation among erythrocytes from humans, horses, sheep, and rabbits (40, 98, 172, 179).

The intensity of the hemolysis is variable. Reports of total loss of this activity will not however stand up under critical examination. Minor variations in the quality of the medium may already result in changes resembling an error. Definite relationships exist between hemolysis and growth forms, in that smooth forms possess the greatest, intermediate types, less, and rough forms, the least, ability to hemolyze. On solid media, (see above) hemolysis manifests itself by a narrow band, so that a picture is formed similar to that of the diphtheria microorganism. Erythrocytes are also destroyed in liquid media to which blood has been added.

The dissolution of the blood cells is due to a filterable hemolysin, that is formed also in blood-free media, and that attack erythrocytes after filtration through a filter impervious to bacteria. Studies on its chemical structure and its antigenicity are still in progress.

Hemagglutination. In tests of hemagglutinin formation by Listeria it was ascertained that living suspensions were not able to agglutinate washed human erythrocytes (blood groups A, AB, B and 0) or rabbit, guinea pig and sheep red cells (242j). It should be noted in this connection that a thermolabile hemagglutinizing agent has been demonstrated in the microorganism of swine erysipelas, that agglutinates certain types of chicken erythrocytes after three

washings and exhibits antigenic properties (53, 54). Other cultural characteristics -- Among further cultural characteristics that may also play a role in differential diagnosis, the ability of the Listeria to grow in NaCl concentrations of up to 10 percent, and to endure a pH of 9.6. 0.1 percent methylene blue milk is not reduced. On the contrary methylene blue reduction occurs following the addition of it to a bouillon culture that has been incubated for 24 hours. Generally the reductive ability of the Listeria is quite considerable, particularly evident in measurements with many dye indicators, in neutral meat broth bouillon, where values of minus 200 to minus 260 "m V" are attained. The individual strains do not behave exactly alike. (314). On Simmon's citrate agar no growth takes place; and also not on Endo-Leifson- or Wilson-Blair agar. Tellurium concentrations of 0.1 percent do not inhibit most strains. The bacteria multiply well in bromthymol blue milk, and act to acidify the medium without curdling after 2-3 days incubation. Of the characteristic odor we have previously spoken; this acid smell arises also from milk agar and potato-blood-glycerin agar and may cause confusion with Lactobacteria. However, in contrast to this one also always finds with Listeria a positive catalase-reaction. Bile in concentrations up to 40 percent does not influence growth. Good growth results in

infected hen's eggs in the white and yolk without resulting in grossly visible changes in appearance and smell (349a).

Biochemical Behavior. On the basis of its fermentative behavior, L. monocytogens belongs to bacterial groups that show little biochemical activity as it has already been shown that no indol, ammonia or H_2S is formed, that they cannot reduce nitrate, and that they do not hydrolyze urea. No proteolytic properties are detectable.

Correspondingly, gelatin and coagulated serum are not liquefied. Splitting of sugars is easy to demonstrate in media with a meat extract-peptone base, and yields typical results. Relatively little carbohydrate is attacked and decomposes with formation of acid without development of gases. Results of research of different authors on a whole series of sugars, alcohols and alcoholic derivatives of sugars show good general agreement, but differ from one another in the case of a few carbohydrates. This sometimes stems from variations in the fermentation apparatus of individual strains, that for example in the decomposition of melezitose have been developed as constant characteristics. In other instances the research results were influenced by the use of different indicators. If indicators with a change-over point near to neutral are used, acid production from the different carbohydrates may be observed, that cannot be detected if indicators with lower, more acid, changeover points are selected.

The following table, which is based on the use of brom-cresol-purple as an acid-base indicator, in 1 percent sugar solution, gives an insight into the sugar splitting capacity of L. monocytogenes.

Table I. Sugar splitting of L. monocytogenes (tested 1951-1953 on 55 strains).

Highly acidified in 24-72 hours	Limited acid formation	No acid formation
Dextrose	Saccharose	Mannitol
Maltose	Glycerin	Lactose, (some of the strains)
d-levulose	Sorbitol	Dulcitol
Salicin	Xylose (irregularly)	Starch
Esculin	Melezitose (some of the strains)	Adonitol
Dextrin		Inositol
Trehalose	Lactose (some of the strains)	Arabinose
Rhamnose		Raffinose
Melezitose (some of the strains)		Inulin
		Melezitose (some of the strains)

Occasionally strains are reported in which the given reactions vary; for example, saccharose after 24 hours will be decomposed, with

strong acid formation (99) or lactose will be swiftly decomposed (253c). In general, the views on lactose splitting by Listeria are divided. Often a more or less strong acid formation is indicated, in the case of many strains only after many weeks ((40, 172, 179a, f, 194a, 240). Other researchers came constantly to a negative conclusion (132a, 204a, e). As the reaction results undergo certain limited variations in observations conducted over a period of many years, which may no doubt be explained in connection with certain minor changes in composition of the medium, both views may be correct. It is feasible to test for the sugar decomposition with relatively coarse and rough methods, as has been superseded by many other diagnostically useful criteria. Among a material consisting of over 200 strains of Listeria, the findings of our studies yielded no culture that was capable of swiftly decomposing lactose.

Similarly with the decomposition of galactose, which, in contradiction of numerous reports of a series of authors (84, 232b, 323) could not be demonstrated.

Certain exceptions appear also with respect to the splitting of xylose (Summary(258)).

In testing for melezitose fermentation clear results are obtained only through the use of brom-cresol-purple as an indicator. If on the contrary bromthymol blue or phenol are used, differences arise.

According to Nyfeldt mannose and amygdaline are quickly decomposed while arbutin is only irregularly attacked and erythrite is often not decomposed at all.

In dextrose decomposition important intermediary metabolic products are found such as acetoin, (acetyl-methyl-carbinol). While the error of acetoin formation has been noted separately (98,179, 298, et al) we found (2421) in confirmation of the findings of others (15,132a,202) the Voges-Proskauer-reaction to be strongly positive, if cultures were tested according to the technique of Barritt or O'Meara. Listeria are active in the formation of acetoin, however, positive results are only obtained when the more sensitive test techniques are used (compare 161). On the contrary, the methyl-red test is always negative.

3. Serological Characteristics.

Since the middle (1930's people have been increasingly occupied with the antigenic structure of L. monocytogenes and have come to the conclusion that the antigenic structure is not uniform.

a) Agglutination reaction.

Seastone had increased our knowledge by the first studies with a large number of strains of both human and animal origin (sheep, cattle, chicken). He attained titers of 1:2000 to 1:10,000 in immune

sera and found in agglutination and absorption studies a close relationship of cultures isolated from man and animals with the exception of B. monocytogenes, which reacted differently, serologically.

Carey, (1936) established the identity of a strain isolated from a child with that cultured by Seastone in his American studies, but saw a completely different antigenic behavior in the original strain of Murray, Webb, and Swann.

Webb and Barber (1937) reported on far-reaching similarities in serological behavior of strains 58.XXIII, 53.XXIII, Pirie, Schultz and Gibson; and in that year Schultz and his coworkers confirmed anew the differences between the cultures isolated in New England by Burn and Ten Broeck, from the British strain as well as from one of his own cultures. Porzecanski and de Baygorria mention that the strain they found in Uruguay was similar to that found in North America by Seastone.

Exact conclusions were first brought about by the studies of Julianelle and Pons who detected two clearly distinct serologic groups. Since in the group of predominantly rodent strains (rodent group) and in the other group on the contrary predominantly ruminant strains (ruminant group) were found in such an obvious relationship in connection with the source of the strain and its type-classification, that it is further reflected in the naming of both groups. These

distinctions are often incorporated into the latest textbooks. Of importance was the finding that Listeria cultures isolated from man demonstrated no special position, but could be classified as belonging to either or the other of the two groups at various times.

Outstanding conclusions with respect to classification of types and positions in animals give rise to contradictions as Patterson found in extensive studies (190 a,b) with 54 strains of Listeria with the method so successfully used in Salmonella serology, for the exact analysis of the body (O) and flagella (H) antigen (White, Kauffman), which he employed here for the clarification of the antigenic structure of L. monocytogenes.

Success was had here in differentiating between various somatic antigenic factors that made subdivisions in the O-group possible. However, since the H-antigen is also composed of many partly overlapping, partly strongly specific antigen components, altogether four serotypes may be differentiated, that may be assembled into an antigen pattern (Table 2).

Table 2. Antigen structure and classification of types of L. monocytogenes (after Patterson).

Type	Flagella antigen	Body antigen	No. of strains	Host	Example	Source i.e. Nation
1	AB	I II (III)	20	Rodent Man Fowl	58.XXIII Schultz LS/2	England USA England
2	BD	I II (III)	1	Man	Gibson	Scotland
3	AB	II IV (III)	10	Man	N/46	Denmark
4	ABC	V (III)	23	Ruminants Fowls Fox Man	J&L G 101 LS/13 Ten Broeck B9227 Cotoni Burn(?) Montevideo	USA New Zealand England USA USA France USA Uruguay

Paterson summarized the results of his research as follows:

"There does not appear to be any relationship between the bacteriological type and the zoological host species, nor do the types appear to be associated with a particular geographical origin. It will be noted that members of each type have been isolated from man and that poultry strains fall into types I and 4."

The serologic findings were later confirmed and broadened with the detection of additional antigenic factors (293). Robbins and Griffin in 1945 came to similar conclusions, besides which

they studied the action of a disinfectant upon the flagella antigen as well as the effect of heat and alcohol on the O-antigen of Listeria and in connection with this studied the antibody formation against single components of the antigenic complex. The results are of great significance for all concerned with the serology of the Listeria, and therefore ought to be specially mentioned.

Among the O-antigens the factors I, II, IV, and V proved to be thermostable, that is, they survived heating for many hours to 100°C. and, also, their antigenicity was not influenced by high concentrations of alcohol. In addition, body antigen III was also found in our own, later research to be quite volatile (242).

The ability to form agglutinin was lost in some, but not all strains on previous treatment with heat or alcohol, for the III-antibody; but not the ability to agglutinate, and according to our own research, also not the ability to form agglutinin. Robbins and Griffin view the source of the variability of Factor III as being due to its chemical structure, but also to the arrangement of and the distribution of the III molecule in the bacterial soma. Perhaps it is here a question of a surface antibody of the type of the L-antigen of the Enterobacteraceae. The knowledge of the variability of the III antigen gives the explanation for minor variations in O-antigen content of strains of otherwise similar types.

No less important is the selection of a suitable lethal agent for preserving the H-antigen. Phenol is not very satisfactory here, since it enhances spontaneous agglutination, that however, may be prevented by buffering the diluent with m/5 phosphate mixture at pH 7.2. Just as by phenol will Patersons H factor A, also be destroyed by Chloramine T and merthiolate, and H factor C is similarly destroyed by merthiolate (sodium-ethyl-mercurithiosalicylate, Lilly).

On the contrary, formol is also particularly suitable for use with Listeria; H-antigenicity, the ability to agglutinate, and the capacity for agglutinin formation are fully preserved. One must only bear in mind that formalized Listeria antigen is particularly applicable only in testing for H-agglutination with the use of this special method, since in Listeria O-sera a definite inhibition of the agglutination of the formol antigen may be observed.

From these findings it is evident that the technique of antigen preparation in use at the present time has decisive significance for the evaluation of research findings in the serology of Listeria.

One further factor that must be taken into account, is the variable appearance of agglutinin in the serum of research animals during immunization.

This concerns, besides the O-agglutinin III (see above), particularly the H-agglutinin.

While the H-factors A, B, C and D have detectable formation of agglutinin relatively early in the series the appearance of C-agglutinin has been shown to be definitely delayed. In the studies of Robbins and Griffin it appeared first in the second half of the period of immunization, which itself extended over many weeks. We ourselves (242i) were able only after many immunization cycles to isolate C-agglutinin at low titers.

It remains an open question as to whether the so-called C-factor of Paterson, that he could find in all strains of serotype 4, really is an H-agglutinin. Thence the saturation-formula worked out by him that Donker-Voet has lately worked with (339) does not exclude the fact that the O-factor V is wholly incompatible with the remaining titer.

A source of error that is not to be underestimated is the tendency towards spontaneous agglutination, that is often present, and that may be largely eliminated by bathing the antigen in distilled water, through changes in the electrolyte content of the suspending media, but best by shaking for a short period of time.

In our own studies the technique described below has given good results.

Antigen preparation for serum determinations and agglutination.

Twenty-four-hour old cultures grown on buffered 1 percent dextrose agar at 37°C. are suitable for the preparation of O-antigen.

They are placed in physiologic, phosphate-buffered NaCl solution and cooked 1 hour in a Koch kettle to drive off the H-antigen. An end solution of 5 percent phenol is added for preservation. To prevent the formation of masses and the tendency toward spontaneous agglutination which the cultured forms of the strain usually exhibit, all O-antigens are shaken for 5-10 minutes in an Ultrasonator. The frequency and the duration of the shaking is regulated according to the degree of clumping and also upon the thickness of the suspension. At a vibrational frequency of "1mHz" the aim is achieved in 5 or 10 minutes generally with 32-38 watts. The bacterial aggregate is thereby homogenized without any injury resulting from being subjected to ultrasonic vibrations for such a short period of time. The antigen thus manufactured remains stable in suspension and still usable after half a year in a refrigerator.

O-antigen manufactured by the above described method frequently gives an acid reaction despite being absorbed in a buffered NaCl solution. This has no definite effects on immunization, but does on agglutination studies.

The ability to agglutinate spontaneously increases with rising acidity. Therefore for this purpose (eliminating spontaneous agglutination) a careful neutralization to establish a pH of 7.2 is absolutely necessary.

H-antigen is obtained through the addition of equal volumes of

0.6 percent formol-NaCl solution to an 18-24 hour old culture on dextrose bouillon that was incubated at room temperature and was phosphate buffered, after which by microscopic observation the extent of error of characteristic colored forms was determined for the rough forms, and their maximum mobility was determined in hanging drops. Such H antigen is not always very agglutinable. More suitable are H-antigens from bacterial plates after the method of Gard. The medium is modified by the addition of 1 percent dextrose and phosphate buffer mixture, and its surface must be moist, so that if necessary its surface must be moistened with dextrose bouillon. Then, the bacteria are dropped into the medium and after 72 hours growth at room temperature are rinsed with 0.3 percent formol--NaCl solution. 1 percent dextrose agar plates moistened with an agar concentration of 1.5 percent fulfill the same purpose.

Serum is obtained by inoculating rabbits intravenously at intervals of 3-5 days with increasing quantities of antigen, beginning with 1 ml. When a high enough titer is obtained, whereupon it can be found in blood withdrawn regularly at the end of each week, immunization is discontinued and the serum is obtained by heart puncture or exsanguination. After the addition of a disinfectant, in a refrigerator at 4°C. O-sera will be best preserved by the use

of phenol in an end concentration of 0.5 percent, and H-serums by 50 percent glycerine or merthiolate at a dilution of 1:10,000. Before immunizing, it is always necessary to ascertain whether or not the serum of the animal contains Listeria agglutinin. If these are present in titers of 1:80 or over, that animal cannot be used for the obtaining of Listeria serum.

It is easy to further the manufacture of OH sera by injection of formolized bouillon cultures or agar eluates. Naturally, living bacteria may also be used here. But then one must take into account the loss of the animals. It has been overlooked so far, that nothing will be gained by immunizing with living Listeria.

Pure O-sera is obtained by the injection of eluates that were boiled for 30 min. to 1 hour. In practice, for the analysis of O-antigens OH-sera may be used, if the flagella antigen is previously destroyed by boiling.

Pure H-antigen may be obtained by the saturation of OH-serums with O-antigen of the strain being used for immunization. Hirato and coworkers (111) used for immunization shaken Listeria eluates, from which the bodies of the bacteria had been drawn off by centrifuging and which contained the broken off flagella in great quantities. This antigen yields nearly pure H-sera.

Methods of eluation:

1. Use of O-antigens.

For this purpose Drogalski plates containing 1 percent dextrose agar that is phosphate buffered, are inoculated with L. monocytogenes from an 18-24 hour old bouillon culture and incubated for 24 hours with the thermostat set at 37°C. The growth is placed in sterile phosphate NaCl solution, cooked one hour, fixed by the addition of phenol-NaCl and centrifuged. The overlying liquid is drawn off and the precipitate used for absorption. For this the precipitate is mixed with a corresponding quantity of 1:20 or 1:40 dilution of OH serum and incubates this mixture 18 hours in a water bath at 50°C. Even if after this time the mixture is still quite cloudy it is recommended that the neutralization be repeated in a second stage of work. Thenthe pure H-serum may be cleared of any remaining particles of bacteria that are still in suspension and preserved by the addition of 0.5 percent phenol. This kind of H-serum may be kept for a year.

In the same way the O-antigens of different types are obtained and used for the manufacture of O-factor sera.

Empirically it was found that in the case of immune sera with high O- and H-titers at the least the growth of 3 to 5 Drogalski plates are required for the removal of the homologous O-agglutinin from each 0.1 milliliter of whole serum. These figures serve only

as a stopping point; as naturally they vary with respect to the serum titer and the quantity of bacteria.

2. Use of H-antigens.

The growth of surface cultures on phosphate buffered 1 percent dextrose agar in Kolle dishes after standing 18-24 hours at room temperature serve as H antigens for obtaining H factor sera. It should be remembered that the surface of the medium should be as damp as possible and that only cultures showing maximal motility be inoculated. The bacteria are then placed in 0.3 percent formol-NaCl solution and softened with the thermostat at 37°C. then thoroughly centrifuged, and used as the sediment in the second absorption, that is obtained in the same way as described above, at 50°C.

The H factor sera, when preserved with phenol, may also be kept for a full year.

Research techniques in agglutination studies.

For study, the antigen should be so placed in tubes that about 500-1000 million bacteria are contained in 1 ml. of the eluate when ready for use. The standardization is made considerably easier by the use of a barium sulfate standard after MacFarland.

1/2 ml. of the antigen eluate will be added to the same quantity of the dilution of serum under consideration, beginning

at 1:10. 0.85 percent of phosphate buffered NaCl solution serves as a means of suspension in all cases, according to the technique inaugurated by Hutner, since thereby the tendency toward spontaneous agglutination that is often present is reduced to an absolute minimum. The dilution of the NaCl concentration to 0.2 percent recommended by Boeckels hereby becomes superfluous. (242c, 18).

For carrying out the serologic research on reagent glass, it is necessary to read off O-agglutinations after 14-18 hours stay in waterbath at 50-52°C., followed by a few hours stay in a refrigerator at 4°C., and including the usual antigen and serum controls; they are best read with the naked eye against a dark background and with the light coming from the side. For use of the agglutinoscope the control tube with antigen-NaCl mixture must always be available for comparison.

The agglutinates are lumpy in the lower serum dilutions, shredded or sausagelike. At higher dilutions they are fine, granular, and can not be shaken up. H agglutination may already after 2-4 hours (the latter time for H-factor sera) stay in a water bath at 50°C. followed by 15-30 minutes standing at room temperature. Here the agglutinoscope and magnifying glass are best set aside, and the results read by the naked eye in suitable illumination. H-agglutinations are recognized by a white, flaky precipitate and can be easily shaken up. That which cannot be clearly seen as H-agglutination by the naked eye should not be considered as such.

The study may be carried out on an object glass:

It is more feasible here to use a somewhat thick antigen. The O-antigen submitted to sound waves is centrifuged and the supernatant liquid drawn off until a small residue remains in which the precipitate is again suspended. A drop of this eluate is mixed with a drop of O-factor serum on an object glass and after keeping on a slant for 2 minutes is observed with appropriate lighting. For a most appropriate test one may place simultaneously on an object glass an antigen in two or three factor sera and a drop of NaCl solution for control. In this way one has at once serum and antigen controls before his eyes and obtains greater certainty in reading and in evaluation.

Not so simple is the object glass agglutination in testing for H-antigen because the preparation of good flagellate antigen suspensions from surface cultures is not usually possible. The author used 18-24 hour old cultures, which were grown on the surface of moist, buffered 1 percent dextrose agar with an agar concentration of 0.8 to 1 percent at a temperature of 22-24°. If also from most strains an outgrowth analogous to the Salmonella was absent from the Gard dishes, there results, however a sufficient development of flagella, to make possible the agglutination research with H whole and factor sera with living antigens. With a small platinum loop or the point of a platinum needle one takes some of this bacterial

material up, from the surface of such a colony according, naturally, as far as possible a growing area, and dissolves this carefully in a drop of H-serum previously placed on the object glass, and, for control, into a drop of NaCl solution. A valid, flaky agglutination is only demonstrated if the control reacts unequivocally negative.

With the four Listeria strain types of Paterson that are preserved in the National Collection of Type Cultures, London, (Dr. Cowan) in the Institute of Bacteriology of the University of Lousanne (Dr. P. Hauduroy) as well as in the Health Institute of Bonn University, may, in the following manner, by the use of the previously described technical procedures, be determined with serum suitable for detection of types.

(see table 3 and 4 on next page)

In our own studies the agglutination formulas developed by Paterson were in general confirmed. They are found useful for quick determination of Listeria types on a serologic basis and they may be simplified even more for routine investigations, so that the following diagnostic formulas may be formed (Table 4).

Every strain that is agglutinated by this O-antigen in I, II serum and I serum, belongs to type I or type 2, testing of H-antigens in sera A, B, and D, making possible the type diagnosis.

Table 3. Absorption formulas for Listeria O- and H- factor sera.

O-factor serum[1]	I, II	O-serum from No. 7973, saturated with No. 5214 or any other type 4 strain.
	I	O-serum from No. 7973, saturated with No. 5105 or any type 3 strain.
	IV	O-serum from No. 5105, saturated with No. 7973 or any other type I or type II strain.
	V	O-serum from No. 5214, saturated with No. 7973 or any other type 1 or type 2 strain.
H-factor serum	AB	OH-serum from No. 7973, saturated with O-antigen of No. 7973.
	A	OH-serum from No. 7973, saturated with O-antigen of No. 7973 and H-antigen of No. 5348, or OH-serum No. 7973, saturated with OH-antigen from No. 5348.
	D	OH-serum of No. 5348, saturated with O-antigen of No. 5348 and H-antigen of No. 7973, or OH-serum of No. 5348, saturated with OH-antigen of No. 7973
	C[2]	OH-serum of No. 5214, saturated with O-antigen of No. 5214, and H- or OH-antigen of No. 7973.

1. With the use of whole sera, H-agglutinins are still present after saturation therefore only O-antigen may be tested.

2. Apparently the C-agglutinin formation is not constant.

Table 4. Simplified diagnostic antigen pattern for L. monocytogenes (Seeliger and Linzenmeier)

Serotype	Original No. of the Strain	Diagnostically important	
		O-factors	H-factors
1	7973	I, II, ...	A, B
2	5348	I, II, ...	B, D
3	5105	II, IV, ...	A, B...
4	5214	V, ...	A, B...

Instructions for carrying out the studies:

O-factor sera I, II-I-IV, and V are needed as well as H-factor sera A--A, B and D.

Strains that are agglutinated by I, II sera but not by I sera belong to type 3. Proof: agglutination in O-serum IV.

Cultures of which the O-antigen is not agglutinated in O-sera I, II and IV but are agglutinated in O-serum V, are to be cleared as type 4.

Type V-sera reacts equally well with all type 4 strains whether they belong to subtype 4 a or subtype 4 b. In addition to a common O-factor, it contains still another agglutinin, that is not removed by saturation with the strain 1071/53 -Preuss, Wuppertal, and only agglutinates strain 4-a. Conversely, one finds in the O-serum of

the strain 1071/53 in addition to the common agglutinin, a receptor, that is not removed by absorption with Paterson's type 4 O-antigen. This O-factor serum agglutinates only 4-b strains.

As is obvious, only as small a quantity of pure H-serum may be developed as of pure B-serum. They are however, unnecessary for practical diagnosis, for the same reasons as C sera.

With these factor sera determined for routine diagnosis, Seeliger (242 d,j) typed more than 200 Listeria cultures from all parts of the world. Among them were 50 strains freshly isolated during the years 1951-1954 from human cases of listeriosis in Germany.

The results set forth below in Table 8 (Footnote: pages 56-57 of the text.) show that generalizations--perhaps in the sense, that O-group I (types I and 2) are designated as rodent groups and O-group 3 (type 4) as ruminant groups--are untenable and even misleading.

Overlapping serologic reactions between the Listeria serotypes and other kinds of bacteria have to date only exceptionally occurred.

Studies carried out for this purpose (15, 132b, 242i) always demonstrated the serologic differences between L. monocytogenes and various strains of the Erysipelothrix--group. Besides, no relation

to Lactobacilli or Acidobacteria can be demonstrated. Perhaps further research is needed here, as Ludenkaemper, in a strain that we regarded as belonging to the Listeria group, found strong cross agglutination in Acidobacteria serum. According to Oezgen, there is also no relation to Corynebacteria and other gram-positive, motile, or immobile bacilli. However, the writer observed that Enterococci, could be strongly agglutinated in Listeria type 4 sera, but not in type 1, 2, and 3 sera. Other Enterococci strains showed genuine O-antigen similarities with types 1-3 of L. monocytogenes; (see p. 121, 127)). Overlapping has also been found between B. coli and Listeria (123, 319).

These findings remind us to be cautious. Serologic diagnosis alone is not sufficient for diagnosis of diseases due to microorganisms, but they are a reliable aid only in connection with cultural and biochemical methods. The changeover into the cultural rough form is often, but not always, coupled with the loss of the O-antigens. A few strains however, possess the whole O-antigen, although they grow as rough forms without exception. Type 4 strains change over more often, according to our own research, into the serologic R-form than do strains of the remaining types. In this state they are, whether living or cooked, no longer or only with great difficulty agglutinable in type V. serum. The agglutination is weak or sporadic only and will be detectable after 18 hours at 50°C., to be sure, at low titers.

b) Precipitation reaction.

The findings obtained by means of the agglutination reactions of the different serogroups or types may be confirmed by the results of the precipitation reaction.

The fine diagnosis of H-antigen components is generally omitted here. The usual methods for obtaining bacterial extract almost always destroy the sensitive proteins, of which the flagella are composed, and spare thermostable water soluble substances that are precipitable by acetone or alcohol, which will give positive reactions to the Molisch test and will yield negative protein reactions. Accordingly these have polysaccharide characteristics, which consist of the polysaccharide fraction of the O-antigen, as the precipitation reaction shows.

Method and technique may be assumed to be known. In principle, the procedure is always the same:

Capillary or test tubes (0.1-0.5 cm. diameter) the first filled with immune serum (if necessary in suitable dilution) and carefully covered with a layer of the extract or extract dilution. Readings are made after 30 minutes at room temperature and after further 18 hours stay in a refrigerator. Formation of a definite white ring at the surface limits and subsequent settling of a white sediment in the cup of the tube are valid indications of a positive reaction.

An elegant method of making visible the antigen relationships consists of precipitation in agar gel, that, with respect to the methods of Oucherlony and others, may be used in the study of the antigen structure of individual bacterial species (242 j,m).

High quality immune sera are prerequisite for unequivocal results.

Filtrates of old bouillon cultures serve as antigens (132 a, b, 204 e) as do extracts of agar cultures that have been shaken for many hours (188 a), polysaccharide extracts according to Fuller (56, 242 l), or according to Boivin and Mesrobeanu (242 c, i).

The findings correspond to many respects to those of O-antigen formulas and may be perceived as three different groups according to the polysaccharide-precipitinogen content (56, 242 i).

In general, the extracts react more specifically than the total antigen in agglutination study. The cross relationships between the individual types that are detectable by agglutination tests are barely perceptible in precipitation reactions. Only the O-identical types 1 and 2 behave the same. While the immune sera of type 3 also precipitates extracts of types 1 and 2, the precipitinogen of type 3 reacts only in homologous serum. Sera against type 4 precipitate only feebly extracts of types 1 or 2. These concomitant reactions arise also in agar-gel study, (Figure 11).

Figure 11. Precipitation in Agar-gel (242 m) with L. monocytogenes type 4 serum.
Middle dish: O-serum of L. monocytogenes, type 4
Left upper dish: Polysaccharide from L. monocytogenes type 4, Paterson strain.
Right upper dish: Polysaccharide from L. monocytogenes type 4, Strain 8/55.
Left lower dish: Polysaccharide from L. monocytogenes type 1, Paterson.
Right lower dish: Sodium chloride solution.

As concerns other kinds of bacteria the opposite is true.

The cross reactions with streptococcal or enterococcal antigens that are present in agglutination and complement fixation reactions are only partially confirmed in precipitation tests. While, for example, Listeria sera of types 1 and 2 precipitate the polysaccharide fraction of a known strain of enterococci (No. 98) strongly, conversely there is no such precipitation of Listeria polysaccharide fraction in hyperimmune serum of the streptococcus strain No. 98, although this strongly flakes out the homologous precipitinogen (242 j).

Extracts of six different kinds of bacteria were not precipitated in Listeria sera; conversely, precipitinogen of the Listeria type was not precipitated in antisera from streptococcus, typhus and Flexner bacteria (56). Two sera of the Nyfeldt and Gibson strain precipitated, however, the carbohydrate fraction of hemolyzed staphylococci, so that the possibility of further cross-reactions must be reckoned with (56).

Later works of Jaeger and Myers (123, 319) deal with the L-antigens (Kauffmann) of L. monocytogenes.

It was found thereby that the Listeria extracts containing lipoid after extraction of the organic matter with chloroform and ether may be precipitated by L-antisera from E coli K 8 (0 8). From this it was concluded the the L-antigen is a component of the extractable lipoids. The L-titer decreases with increasing age of the culture.

4. Listeria types.

The widespread cultural and biochemical individuality of L. monocytogenes contrast thus with a manysided serologic picture that finds its parallel in the Salmonella, Pasteurella, Leptospira, and Brucella--groups.

After repeated observation of Listeria strains over a period of now nearly 30 years by different research workers with results equivalent in principle, it may be definitely said today, that the serologic Listeria-types are constant in vitro.

Doubtless the number of serologic types that are known does not yet include all the types that are actually present and are serologically differentiable. Because initially, the antigen formulas simply express only qualitative differences, and allow quantitative differences to be disregarded, such as for example are found in the antigen analysis

of Brucella to be most important characteristic. There are indications that such quantitative differences exist also in the case of Listeria (204c,2421,j, 281b). Their observation would so complicate the serology of Listeria that man has given up further research in that direction.

There are, however, still qualitative differences in the antigen content of the individual types. Thus there exists, by way of example, after the studies of the author and of Donker-Voet, concerning serotype 4 at least two subtypes divisible with serum factors, whereupon Paterson's type strain corresponds to subtype 4 a and the new type strain (Preuss-Wuppertal 1071/53) to subtype 4 b. The preponderant number of type 4 strains tested to date belong to subtype 4 b (242j).

As already explained, there is no definite relationship between the bacilli and host or source of origin (country in which found) and the serologic type on the other hand. It is certainly no accident that the overwhelming majority of the rodent strains belong to type I and that most of the strains from larger animals belong to type 4.

However this division that was initially so sharp is increasingly effaced with extension of the studies. Gudkowa and Sacharoff report on changes in Listeria--serotypes in the animal body, for example, on the development of a special horse serotype from the rodent type, etc. Such findings have not, to date, been confirmed. We view them, just as we do the conclusions derived from them, (345) with extreme skepticism.

--In any case, the serologic type determination of Listeria remains a helpful means of clarifying epizootiologic and epidemiologic relationships if one does not wish to limit the determination of the source of infection in Listeria to mere conjecture.

Table 5. Melezitose fermentation in 162 serologically analyzed Listeria strains and the source of the same (duration of observation: 5 days at 37°C. and 4 weeks at 22°C.)

Serotype	Melezitose-fermentation	Number of strains	Habitat	Source (Country)
1	negative	70	Humans, rodents fowls, cattle, canaries, hares, guinea pigs, sheep	England, South Africa U.S.A., Sweden, Canada, Germany, Israel, France
	much delayed positive	10	Humans, cattle horses, rodents, fowl	Germany, England Canada
	positive after 2-4 days	19	Humans, rodents, fowl, cattle, sheep, deer	Australia, Sweden, England, Canada, the Arctic, U.S.A., Germany, Austria, France
2	negative	1	Human	Scotland
3	negative	6	Humans, sheep	Denmark, Germany
	much delayed positive	1	Human	Denmark
4	negative	7	Humans, fowl, hares	Canada, U.S.A., France
	much delayed positive	3	Human, sheep, fowl	France, U.S.A.
	positive after 2-4 days	45	Human, cattle, sheep, pigs, ferrets, goats horses	U.S.A., England, Germany, France, New Zealand, Argentina, Japan, Israel.

Subdivision into biotypes serves the same purpose. With the similarities in fermentation activity, of L. monocytogenes, only that for melezitose is useful here.

Harvey and Faber found, in 1941, a noteworthy relation between melezitose fermentation and serologic type classification. With the general validity of this finding it would perhaps be possible to generally replace the usual serologic type by biotype determination. To answer this question we undertook the study of 162 strains, which yielded, when a 1 percent melezitose--bouillon with bromcresolpurple as an indicator were used, no relationship between sero- and bio-types of L. monocytogenes (242d,k).

Substitution of seroanalysis with biotyping is not thus possible, but it can function as a supplementary aid.

A regional distribution of the types is detectable.

In Canada as in the USA are present mixtures of types 1 and 4 but also regional increases in either one of the two types. In Germany, on the contrary, strains of type 4 are found much less often than type 1 (242i,j) while in France 242k) and Holland (339) types 1 and 4 are found just about in equal numbers. Incidentally, type 3 has to date been identified only in Denmark and Pomerania (190b, 242j) and lately in Canada too; the only type 2 strain known to date was isolated in Scotland (190b).

Whether there are also phagotypes of L. monocytogenes is to date not known. Studies being conducted in the USA at present promise further findings in this respect in the forseeable future. To our knowledge no lysogenic Listeria strain or specific bacteriophage has yet been detected.

The nomenclature of the serotypes has been established since the studies of Paterson, and these formulas and type designations have been repeatedly confirmed (56,214,2421,293). The Japanese serotypes set forth by Hirato and coworkers in 1953 are, according to our own research (242j) to be identified with Paterson's types 1 and 4; and, specifically, type A (Hirato, et al.) corresponds to serotype 1 and type B and C, (Hirato, et al.) to the serotype 4.

5. Capabilities for causing monocytosis.

Notwithstanding the serologic complexity and biochemical differences all sero- and biotypes of the species L. monocytogenes are united in their ability to cause monocytosis. This property is so typical that it has been adopted as the name for the species.

Intravenous injection of living Listeria results, in rabbits, in a definite increase in monocytes in the peripheral blood count on the 3rd or 4th day postinfection.

Murray, Webb, and Swann emulsified agar suspension by strong shaking with glass beads in physiologic NaCl solution and dispensed

them as intravenous solutions, which were calculated in mg. of wet weight, according to the body weight of the rabbit in kg. Careful predeterminations were necessary to obtain the correct infection dose. It must be such that the animals do not succumb to it during the first week of observation. This is achieved near the LD_{50} (Footnote: By LD_{50} is meant the infectious dose that will kill 50% of the infected animals (calculated according to Reed and Münch's method; Am. J. Hyg.: 27, 493 (1938))). With too small an inoculum the infection does not progress, and thus no monocytogenic activity results. Here, also, the proper research technique is indispensable for good results, and the failure to observe methodical procedures must lead to false conclusions.

Not all research animals are capable in the same fashion, of reacting to a Listeria infection with a definite monocytosis. As a fairly constant rule it may be stated that increased monocyte production arises chiefly in rodents (in natural conditions, incidentally, also in humans) but in larger animals practically never.

Naturally the blood count changes are also subject to certain biological variations, that are connected with the virulence and cultural phases of the strain.

The increase in monocytes observed at Cambridge (172) showed a rise from 420 to 6820 or from 230 to 15,100 cells per cubic millimeter. --

Typical research protocol may be illustrated by the following table:

Table 6. Leucocytic reaction in experimental Listeria infection of rabbits with the Gibson strain from fatal meningitis (excerpted from Webb and Barber, 1937)

Dose; 0.1 mg. per kg. of rabbit	Total number of leucocytes per mm^3	Polymorphonuclears		Lymphocytes		Monocytes	
		%	Number	%	Number	%	Number
Differential blood count before the injection	9,800	32	3135	65	6370	3	295
After 65 hours	13,100	47	6155	39	5110	14	1835
After 96 hours	12,900	37	4775	33	4255	30	3870
After 120 hours	13,800	39	5380	41	5660	20	2760
After 17 days	10,300	36	3705	58	5975	6	620

Often some of the monocytoid cells are difficult to differentiate. Such cells, the "borderline cells" of English authors, have been viewed many times as transitional cells between monocytes and lymphocytes. Nyfeldt and also Bloom believe that these monocytoid cells that on the fourth day after infection comprise 21% of all leucocytes (compared to 21% granulocytes, 1.5% lymphoblasts, 27% lymphocytes, and 28% monocytes) (179f) are products of the lymphatic system. The differentiation of this kind of transitional form is extraordinarily difficult and is the subject of exhaustive research. (33,45,179a,b,b,195,211,269,291).

During the monocytic phase an increase in basophil cells occasionally occurs (124,157c,368). In Nyfeldt's protocol the post-infection percentage in rabbits varied between 0 and 4%. The significence of this remarkable phenomenon is not yet clear.

The blood of infected animals is often sterile. Only in the last stages of fatal Listeria infections does one find numerous monocytes in Giemsa-preparations of the peripheral blood just as in smears from organs and reticuloendothelial cells with phagocytized Listeria (179f,320,194,202,240), so that they may be confused with plasma cells (308, 242).

The authors saw in Nyfeldt's laboratory blood smears from rabbits ill with listeriosis with 75% monocytes and similar cells--among which Listeria were prolifically interspersed intracellularly--that deceptively resembled plasma cells.

During the course of acute purulent inflammations (for example, meningitis) Listeria were, however, also taken up by granulocytes.

Seastone observed the same characteristic increase in monocytes in chickens and guinea pigs.

Of course, other bacteria are capable of causing monocytosis in research animals, for example, erysipelas bacteria (E. rhusiopathiae) (15,60).

Julianelle found in his monocytosis studies a noteworthy variation

and observed cases in which the polymorphonuclear cells increased instead of the monocytes; occasionally it amounted to severe leukopenia instead of the leucocytosis that would normally appear.

No monocytosis can be induced in rabbits by repeated injections of killed Listeria suspension. In humans, too, the injection of such vaccins shows no results with respect to monocyte multiplication (179f).

Stanley (253a,b) achieved similar results; since he found that the injection of 0.5 ml of an 18 hour old bouillon culture of living Listeria usually resulted in an increase of monocytes after 96 hours of from 3 to 24%, and after the injection of a billion (one thousand million, here) of bacilli killed by heat an increase of only from 4 to 9% after 5 days.

The agent that produces monocytosis (MPA) was obtained by Stanley in 1948 while working with Listeria, in the extract from dried and

Figure 12. Reticuloendothelial cells with phagocytized Listeria (mouse spleen) May-Grunewald-Giemsa-staining (after Hervier, Lavergneu

pulverized organisms which had been treated with lipoid solvents. The yield of lipoid extracted with petroleum ether was greatest, but chloroform or ether extracts were biologically more active. The polysaccharide and protein fractions had, in animal research, practically no monocytogenic effect; on the contrary, the monocyte component rose after an injection of 0.5 ml. chloroform-extract, from 2 to 22% after 72 hours.

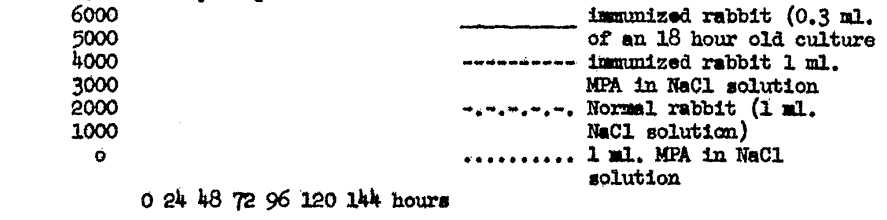
The MPA is found in minute quantities in the wash water and will only be set free after mechanical destruction of the bacterial cell, so that this may, doubtless, serve to explain the negative findings after injection of killed, but otherwise intact, bacteria.

In this connection it should be noted that other lipoids too--for example the fatty acid fraction of phosphatides isolated from tubercle bacilli--have a monocytogenic action in human and animal tissues.

The existence of and properties of the Listeria lipoids were confirmed in Murray's Institute (317).

The most important property of this substance that has not yet been obtained in a chemically pure form is its ability, to cause, in normal rabbits as well as in those immunized with Listeria suspensions, a reaction of the hematopoietic system, that cannot be differentiated quantitatively or in the course of time from experimental monocytosis after infection with living bacteria.

Figure 13. Monocyte curves after injection of living Listeria and of MPA in normal and immunized rabbits (after Stanley (1949)).



In agglutination, precipitation, and complement fixation tests on whole serum from intact organisms, the MPA appears to be not agglutinable, precipitable, complement fixing or antigen-binding, thus, to be altogether inactive.

Later studies (123,319), state that the serologically active L-antigen reacts in similar fashion as a part of the lipoid complexes and can be precipitated by specifically absorbed sera from E. coli K 8 (0 8).

The Listeria-lipoid, although itself not an antigen, increases, nevertheless even as do lipoids from fungi, tubercle bacilli, etc, the formation of antibodies against heterologous bacterial antigens if it is injected concomitantly with them (83b,253b).

A toxic effect is not detectable even after intravenous injection of 5 ml. of a thick lipoid emulsion. In contrast to this the polysaccharide fractions were highly toxic for mice and rabbits. On administration of greater quantities of polysaccharide fractions than the LD_{50} a severe leukopenia develops and the animals die in a few hours without resulting in a rise in the granulocyte count. The MPA will be set free from the bacteria in the liver of animals with a fatal infection. The number of liver necroses (see the following section) parallels the amount of lipoid bodies set free.

The ability to cause monocytosis that varies from stain to

strain will thereby be explained, as the MPA is present only in limited amounts in rough cultures, and its content in fresh strains rapidly declines after passage on the usual media.--Therefore it is advisable for this kind of research to make a passage first in animals.-- Although this substance is hardly toxic, highly virulent strains contain much, but nonpathogenic cultures only a little, MPA.

6. Pathogenicity for animals (including pathologic anatomy).

Listeria strains freshly isolated from pathogenic material and smooth laboratory cultures are, without exception, pathogenic for animals. This is one of the most important characteristics. Testing of pathogenicity for animals should not be overlooked at the first isolation of a new strain.

They extend themselves to practically all laboratory animals and--as is familiar from the epizootiology of listeriosis--to a large number of wild and domestic animals both small and large that for obvious reasons are only exceptionally employed for laboratory purposes.

The pathogenicity is not conditioned by exotoxin.

The result of animal experimentation is authoritatively influenced by the virulence of the strain used. There are highly virulent strains of which the smallest quantity of bacteria will lead to the death of all the research animals, and others, of which the largest infection dose

will cause no actinn. As already explained in the foregoing section, this depends largely on the content of lipoid substance (253a) and will moreover, be determined by the applied quantity of polysaccharide, that may correspond to the endotoxin. Therefore the rough strains when they are lipoid free and polysaccharide poor, are only slightly or not at all pathogenic.

By animal passages definite increases in virulence may be obtained.

Of critical significance are the infection dosages (172, 281b) and the method of application. By using doses less than the LD_{50} an increasingly greater percentage of the animals survive the infection. The intravenous, intraarterial and intraperitoneal passages lead most certainly to fatal disease, just as do intracerebral or intrathecal infection. Subcutaneous, intramuscular or peroral administration are fraught with an increasingly larger quota of failures.

Obviously the pathogenicity is different for the various research animals, whereas with many known strains even susceptible kinds of animals cannot be fatally infected. However, there are present, maybe, differences in resistance among the individual research animals.

Research animals: Among the small laboratory animals that are particularly well adapted to experimental Listeria infection are rabbits and mice, which die with great regularity after intravenous, or intraperitoneal, and the latter also after subcutaneous, administration of living bacteria.

The injection of 0.000001 to 0.2 ml of 24 hour old dextrose bouillon cultures led to death in from 1 to 5, but occasionally only after 10-14 days (132a) insofar as the strain had not become avirulent. The existing differences in the doses having lethal action require in controlled studies--about like the determination of the therapeutic index of a drug--a previous determination of the LD_{50}.

It has been reported, furthermore, that chickens (intravenously) (234,240,271), Rhesus monkeys (232), hamsters and golden hamsters (188, 298), sheep (29,82,86c,87,111,134,186 and others), as well as pigs and cows (29,86c,87,248) can be successfully infected.

Zink and coworkers mention besides, that with 12 six-day-old chicks infection did not take place with the strain that they used.

Similar differences in the host susceptibility appear to be present likewise in guinea pigs.

Although numerous authors (31,40,99,132,172,232,234,271 and others) have designated the guinea pig as a suitable research animal, Stanley could induce no infection in one of three animals. The death of infected animals took place on about the 23rd day (19). Other researchers (188,189,242 1) found guinea pigs resistant to tests of strains freshly isolated from humans and animals despite relatively high infection doses.

Obviously larger doses are required to produce infections in

guinea pigs than in other small research animals. Correspondingly it was determined that--in general--only intravenous administration led to fatal infections, while after intraperitoneal administration the animals generally remained healthy (see 294, 322 and others).

Transmission studies were conducted on mountain cocks (155) and doves. Besides, it appears that pigeons are to be considered as often being resistant to Listeria infections, (not, however, against the microorganism of pig erysipelas) (99). On the contrary, it has been reported that horses, dogs and cats cannot be artificially infected (278). Rats will likewise be considerably resistant (188,202). According to still other reports rats are susceptible (278) but relatively large infection doses are needed (281b).

Course of the disease. Just as under natural conditions the course of the disease takes many forms and varies with the host (83). In rabbits, guinea pigs, mice and desert jumping rodents (Tatera lobengulae) the disease is characterized by a generalized infection with massive liver involvement (172, 187 and others) while in chickens, besides the disseminated disease, a severe heart muscle involvement with spreading necrosis (240,271) is noteworthy. In ruminants it is particularly manifested by a central nervous system (CNS) involvement (neurotrophy? see p. 78), that appears as encephalitic or meningitic phenomena. By experimental infection a generalized infection of the

rodent type may also be produced in these animals.

In general, isolated disease of the CNS cannot be induced in small animals by intravenous or intraperitoneal infection, such as are observed particularly in larger animals in their natural state. This is possible in rodents only by direct intracerebral or intrathecal inoculation (40, 240 and others); the same may arise also after instillation of living bacteria into the conjunctival sac, but only, to be sure, after a latent period of many weeks (87,134). Generally the animals are healthy again after surviving a local conjunctivitis. The brain of rodents dying from septic manifestations is bacteriologically almost always free from Listeria, perhaps because the microorganisms found no time, because of the short postinfection duration of life of the animal, to proliferate into the CNS.

Biester and Schwarte observed encephalitis in sheep and pigs only 5 weeks after intercerebral inoculation of brain emulsion of sick animals or after repeated intramuscular injection of living cultures. According to this hypothesis animals might also be fatally infected by the subcutaneous route. While pigs got sick, the older animals survived. Jungherr observed the formation of a hemorrhagic meningitis in sheep after intravenous injection, but after intranasal or conjunctival installation, on the contrary, only a transitory febrile reaction, while in the studies of Olson and coworkers on

sheep only intraarterial injection into the carotid artery was certain to cause it (186a).

A few further examples yield an impression of the importance of the method of application for the induction of pathologic changes.

Urbach and Schabinski successfully produced infections in mice, rabbits, and guinea pigs by inhalation, when the animals were infected according to the technique of Shope.

Intranasal infection led in controlled studies to the formation of a few lung abscesses (40) but remained innocuous in mice, rabbits and monkeys (132,232). By scarification of the oral and nasal mucosa peroral and intranasal infection of mice, goats and sheep was successful, whereby the microorganisms obviously spread along the trigeminal nerve (6,7,82). But similar studies with sheep and goats yielded no results according to other authors (111). In guinea pigs only myocarditis without meningitis resulted from intravenous injection, but after intraperitoneal infection--which had previously been shown to have little results in guinea pigs--the contrary behavior resulted (40). Intravenous infection of pregnant larger animals, e.g., of cows and sheep, is often, but not always, followed by abortion and by a septically infected fetus being born dead (86b,c, 190c). Although the mother animal becomes ill with an endometritis, she does not die of the infection.

Uterine inflammation arises under like conditions also in artificially infected, pregnant rodents. Of course, abortion does not always occur, so that altogether different reports result (86b,c, 95,231,323, and others). With peroral introduction of Listeria strains of different sources by means of sounds or in the mash, Hahnefeld successfully infected pregnant rabbits as well as a pregnant goat, to induce abortion. This was confirmed by Gray (308) who introduced the bacteria in the drinking water or installed them directly into the conjunctiva.

That in artificially infected pregnant guinea pigs a septic spread of the bacteria in the mother animal results, will among other things be shown in that the bacteria can be extracted from the milk glands of such animals that have been rejected.

Alimentary studies on animals that are not pregnant produced no unique results.

Successful studies with mice have been reported repeatedly (132a,b, 234) when bouillon cultures are introduced as the only source of liquid or Listeria is mixed with the mash. On the contrary, negative results (204,242j) have occurred even when the bacteria were introduced directly into the esophagus by means of the probe or mixed in the mash together with milk.

Perhaps in peroral infection the age of the animal plays

a significant role. The bacteria can obviously penetrate the still immature tissues of newborn animals after peroral intake better than the mucosa of fullgrown animals. This explains, for example, why only very young rabbits may be fatally infected by feeding the Listeria cultures in place of water (172). Hunger is also to be counted among the damaging influences (349a), as a fatal listeriosis may be produced in guinea pigs with regularity, by means of infected feed after a long period of hunger.

Eye studies of Anton. To the classical methods of determination of the pathogenicity of L. monocytogenes for animals belongs the production of an experimental keratoconjunctivitis. This test, that was developed in 1934 by Anton and a little later by Morris and Julianelle may be carried out with rabbits and guinea pigs. White mice have also proved suitable (188a).

Technique: After instillation of a drop of a fresh bouillon culture or a suspension of Listeria in the conjunctival sac a purulent conjunctival inflammation develops in 24-48 hours that is often followed by a keratitis. The lids are thick and swollen, from the inner corner of the lid exudes a thick cream-colored exudate that contains, in addition to polymorphonuclear leukocytes, mononuclear cells with numerous phagocytized Listeria. This inflammation generally heals spontaneously, leaving behind them a local immunity; there usually

develops, however, after a few days or weeks, a septic or encephalitic disease, from which the animal then, indeed, may still perish. The eye test is also positive in artificially immunized animals. Julianelle tested a large number of different kinds of bacteria and found thereby that Listeria regularly led to a conjunctival inflammation and that this property could be observed otherwise only with E. rhusiopathiae, and to be sure only exceptionally. If the eye infection with erysipelas bacteria is successful, the animals almost always die.

Transmission to chick embryos. Paterson (190a) infected chicken embryos successfully with Listeria. Eight strains caused organic lesions when injected into the chorio-allantois which were quite similar to those in the liver, heart and CNS in natural listeriosis. The changes in the membranes correspond closely to the picture that is seen in a few virus infections. A lethal dose in 72-96 hours was ascertained by means of a Brown 2-hydrometer, to be 0.1 ml of a 10^{-7} dilution of a suspension. Rough strains caused no tissue changes.

Pathological anatomic changes. The macro- and microscopic tissue changes are largely equivalent in the kinds of animals being studied, or at least are very similar. There are also in practice no peculiarities that would appear to require a special discussion of the pathologic anatomy in natural or experimental listeriosis. Therefore we here shall make a preview of these, concerning what will

be discussed in a later chapter, in order to summarize directly some of the findings in the more important organs.

We are in essential agreement with the statements of the English pathologist Webb, as far as listeriosis in rodents is concerned. In the case of the larger animals we follow the pronouncements of the reports of Pallaske, Biester, and Schwarte, Gray, King, and others. In the scope of this publication it is naturally not possible to refer to all histopathologic changes in detail. Only the more important and most characteristic findings will be included for discussion, that the pathologist encounters in dissection of killed animals and the bacteriologist encounters in his animal studies. Individual items may be read up on afterwards in textbooks or in the references here cited (29,45,86f,87g,146,172,187,189,281b and others).

It is pertinent in experimental listeriosis of small animals, especially rabbits, to note the swiftness and extent to which the histopathologic changes fade away (45). The following phenomena stand out individually:

Peritoneal exudates. The exudate is--if at all present--very rich in cells. In the generally only limited quantity of fluid are found together polymorphonuclear leukocytes and many mononuclear cells, which because of their appearance are designated as monocyte-macrophages. The latter contain many phagocytized Listeria, if the

infection is introduced intraperitoneally.

Mesenteric glands and the omentum. In these organs also the changes are clearly induced only by intraperitoneal infection. They consist of glandular swellings and deposition of swarms of bacteria with monocytes and leukocytes in the fat of the omentum. Histologically focus-like lesions are shown that have an appearance similar to that of the miliary necrosis of the liver described below.

Liver. In all small animals in which the fulminating infection was not immediately fatal; i.e., those that survived the infection for more than 12 hours, characteristic liver changes appeared.

Macroscopically the organ is slightly enlarged and covered with more or less numerous pinpoint-to pinhead-sized gray-white to yellow nodes which gives it a speckled or spotted appearance. The nodules are distributed throughout the whole liver tissue in a miliary manner and after intraperitoneal infection are particularly numerous under the capsule. The organ is of brittle consistency.

In section is revealed a picture of miliary focal necrosis. The foci are formed from small round zones with greatly enmeshed reticulum, in which some large, pallid, mononuclear cells and nuclear debris is incorporated. The latter is more often present in the border zone, where are also found polymorphonuclear leukocytes and lymphocytes

in increasing quantities. In the periphery of the necrotic area the liver tissue has disappeared, all but a few cells. After staining, for example, with the method of Jansen-Claudius-Gram, the foci appear to be bounded by swarms of Listeria. If aggregates containing only a few bacteria are isolated, part of them will be lying free, but others are phagocytized by Kupffer's star-shaped cells.

These sharply defined classifications (of pathologic changes) are sometimes obscured by generalized fatty degeneration.

In principle the same changes--only in increased measure--characterize the pathologic-anatomic picture of liver damage in the septic-granulomatous clinical form of listeriosis among larger animals. These are observed predominantly, but not exclusively, in young animals. Better than by words is the typical picture illustrated by figures 14 and 15, for which we are grateful for the kind cooperation of Dr. Gray, Michigan State College, East Lansing, and the Journal of the American Vet. Med. Assoc.

Spleen. This organ is soft, dark red and enlarged. Focus formation is similar to that in the liver, but the foci are less numerous. In case of infection by mouth the mass of bacteria here in section lie thickly under the capsule, while by hematogenous spread of the bacteria, (intravenous injection), the pulp is most of all affected. The majority of the necroses form at the borders

of the Malpighian bodies and partly encroach upon them. The greater part of the foci consist of mononuclear elements, which are embedded in the pale nucleic network of the splenic pulp. The outer limits are defined by accumulations of Listeria.

Kidneys and adrenals. As a rule these organs are only slightly affected and characteristics of focal structure are not histologically differentiable.

Lungs. Mouse lungs show no changes after intravenous infection in contrast to the lungs of rabbits. The tissue is permeated by multiple gray-white nodes, which are microscopically similar to the necrotic foci in the liver. Only in the center of the necrotic foci do the mononuclear cells clearly stand out. They often show evidence of destructive phenomena, so that a picture is formed which resembles caseous pulmonary tuberculosis.

The nodules often attain a diameter of 1 mm. Giant cell systems of the order of Langerhans cells are detectable in them, which contain phagocytized Listeria; generally one encounters them only relatively seldom. In the border zones of the miliary nodules numerous Listeria are to be found in suitably stained preparations.

Figure 14. Liver of an 8 day old lamb, with white, circumscribed necrotic foci. (With permission of the J. Am. Vet. M. Ass., from a paper by Gray and Coworkers, ibid. 115: 103 (1949))

Figure 15. General view in 100 X magnification of focus-like liver necrosis in cross section preparation. (hematoxylin eosin staining) (With permission of the J. Am. Vet. M. Ass., from a paper by Gray and coworkers, ibid. 115: 103, (1949))

Figure 16. Medulla in a cow with listeriosis; perivascular infiltrate, focal necrosis, neuron degeneration, (hematoxylin-eosin, magnified 500 times (Gray)).

The similarities detected by Webb in the histologic structure of focal pulmonary necrosis in tuberculosis and listeriosis is probably conditioned by the fact that under the action of certain higher fatty acids from waxes and lipoids a generally similar tissue reaction ensues, that is characterized by the tubercle or necrotic focus with numerous mononuclear cells (epitheloid cells). By analogy, the specifically active substance might be isolated from M. tuberculosis and also from L. monocytogenes.

Heart. In rodents only small, infarct-like necrotic foci with aggregations of Listeria are found. In fowls, on the contrary, the heart muscle involvement is predominant (76, 240 and others). They are manifested in the form of yellow-white multiple foci, that are formed in destroyed muscle fiber, of which the nuclei are pyknotic. In the center there is necrosis, peripherally there is necrobiosis. The area around the focus shows accumulations of histiocytes, lymphoid cells, and a few polymorphonuclear leukocytes (189).

CNS. While in small animals macroscopic and histologically detectable changes arise experimentally only under certain conditions of research, or are seen in the relatively rare encephalitic illness of the rodents, widespread damage is found in the brains and medullas of dying larger animals. The bacteria proliferate by preference in the brain stem, especially in the pons. Pathologic findings are often not detectable macroscopically, thus making more amazing the changes found in histologic preparations. In the affected regions one finds abundant perivascular infiltration with lympho- and histio-cytes, in addition to cellular infiltrates in the brain substance, that generally consist of polymorphonuclear leukocytes, monocytes, and an insignificant glial infiltration.

In the gray matter the nuclei of the ganglion cells undergo plasma shrinking, the ganglion itself undergoes degeneration of the dendrite, tigrolysis, and pyknosis, nuclear degeneration or nuclear displacement, so that often only a cell shadow is yet detectable. The vascular and parenchymatous infiltrates arise usually only unilaterally in the afflicted brain. The infiltrate becomes necrotic, unites with true abscess formation. Listeria are usually found chiefly in the border zones. -- The changes resemble in many respects those which take place in "louping ill" (29). Changes that are similar in principle take place in the medulla.

In the meninges it results in hemorrhagic exudates with subsequent purulent leptomeningitis. Polymorphonuclear neutrophils are the predominating cell type, with the presence of more or less numerous monocytes.

With regard to changes in the uterus, intestinal mucosa, and other organs one may refer to the work of Osebold and Inouye.

Conclusions.

In summarizing, it may be said that Listeria monocytogenes (Pirie, Murray, et al.).is established as a kind of bacteria definitely delineable by cultural and biochemical methods, that is characterized by the following important attributes:

Gram-positive to gram-variable, non-sporogenic, capsule-less, aerobic and micro-aerophil bacilli with usually peritrichal flagellation; most motility at 20°C.; biochemically little active. Dextrose and a few other carbohydrates are split with acid formation but without any gas being produced; acetone in formed mannitol is not decomposed. On the usual bacteriologic media, sparse growth at temperatures between 4 and 42°C. Formation of beta-hemolysin. Division of colonies into smooth and rough forms with many intermediate stages.

With the appropriate methods of antigen analysis, successful detection of different body- and flagella-antigens, and therewith the subdivision into serotypes, of which four are well known at present.

The strains isolated from men and animals show no differences. Smooth, virulent cultures are capable of causing an experimental monocytosis, which may also be caused by an extractable lipoid fraction. By conjunctival infection a typical keratoconjunctivitis may be called forth in rabbits. Parenteral inoculation leads, in warm-blooded animals, to septic disease with spreading involvement of organs, whereby the liver, spleen and CNS are most often attacked. Peroral administration results in infection mostly only in young or pregnant animals.

In the classification system the bacteria are related to the Corynebacteria and the erysipelas bacteria but are definitely distinguishable from all known kinds. They comprise their own Genus, perhaps even a special Family.

Because of their characteristics they are, however, easy to confuse with bacteria of the kinds named, and as experience shows, also with culturally similar strepto- and entero-cocci. (For more about the differential diagnosis and culture methods see Section D.)

B. Listeriosis in animals.

Rationale of naming. All diseases caused by Listeria are today called "Listeriose" or "Listeriosis". The name Listeriasis is also sometimes used. The earlier common name Listerellosis was abandoned because of the change in name of the bacillus. (page 1). Besides these etiologic disease descriptions, which include the course of the different symptomatic manifestations, there exist further diverse trivial names or descriptive designations, which were coined on the basis of a special leading symptom, or in consideration of exclusively appearing pathologic-anatomical changes. These will be more thoroughly gone over in the following. Concerning this, listeriosis must be differentiated by the above described disease indications, as an etiologically precisely defined unique disease, that with relative similarity of clinical or pathologic--anatomic symptoms being present, originates through different noxa.

I. Geographic distribution.

Possibly, listeriosis was already discovered as a sporadic infectious disease of animals in 1910 in Sweden (115). But the outbreak in England in stables (1923/24) first gave impetus to the careful study of the microorganism and its mode of action (172). The appearance of plague-like infectious disease among South African desert jumping rodents (194a,b) and the observation of outbreaks in

sheep ranches in New Zealand (82) made it apparent that the microorganism was global in distribution. The steadily growing number of reports since then (table 7) from all continents has shown in the meantime, that--similarly as in the case of Leptospirosis, Pasteurellosis, and so on--not only are a great number of different kinds of animals susceptible, and become ill under natural conditions, but that there are, also, no geographic or geomedical limits. It has been reported existing in cold, hot and temperate climates. By the currently increasing knowledge of further susceptible hosts and by the experimentally demonstrable capability of resistance of the Listeria against unfavorable environmental influences it appears a priori improbable that the disease should be confined to only limited areas.

Concentration of pertinent information (for example, on the North American Continent) should not lead to the conclusion that the distribution is increasing or is greater there than it is elsewhere.

As is the case with many other infectious diseases, it happened that in this connection, listeriosis was discovered to be a zoonosis as soon as it was found in man. This development led, in veterinary research in the USA, to a series of information-rich results and paved the way for studies in European and Asiatic nations.

The statement that the distribution of the disease is worldwide among animal strains does not mean that it ought to be found. Our

knowledge of the extent of infection of the most important grazing areas and varieties of animals is still particularly full of gaps. That applies not only to global detection but also to the distribution of the disease within national and continental borders. There is still need for extensive scientific research on a large number of sources, in order that we may grasp the full significance of the extent of the Listeria infection. That despite the widespread distribution of its foci of infection the disease may manifest itself only as small, locally limited outbreaks may be considered today as proved. It is certain that listeriosis was long ago known in animals under the names of other diseases. As examples are to be mentioned the "staggers" of sheep in New Zealand and Germany (82, 189) and the "malignant catarrhal fever" (22a) and probably, too, the "pig grippe" and other diseases of domestic animals.

The results of present studies conducted in the field of veterinary medicine allow one to conclude that the amount of listeriosis known to exist at the present time is only a small fragment of the actual distribution and extent of infection.

Such conclusions may sound surprising after more than 70 years of etiologic study of diseases, particularly as in the case of listeriosis, one is dealing with a bacterial infectious disease, of which the microorganism may be found by bacteriologic methods of

detection. But they are obtained on probability, if the ratio of those found in human bacteriology or human medicine is taken for comparative purposes. Although here the network of bacteriologic research sites is distributed quite unequally throughout the world, and bacteriologic studies should be much more numerous, there can be no doubt that an immense number of cases of illness exist that are traceable to Listeria-infection, that have been totally misdiagnosed for a period of ten years.

Since it is at present still possible to display the world literature on listeriosis as a zoonosis in table form for a review, the following will give a view of the present known geographic distribution and the kind of animals affected (Table 7).

Of special interest remains the possibility that for the study of the individual questions the necessary literature will be found here so that one may swiftly orientate oneself as to the situation up to the fall of 1954.

Table 7. Review of the existence of listeriosis in the animal kingdom.

Country	Province	Year	Kind of Animal	Epidemic	Sporadic	Authors
Australia	Western Aust.	1937	Sheep	+		Gill
Belgium		1952	Chicken	+		Geurden &
		1954	Calf		+	Devos
Brazil		1942	Rats		+	Machiavello
Canada	Ontario	1947	Chinchilla			Kennedy
	Ontario	1949	Chinchilla	+		McKay, et al.
			Calf		+	Fish & Schroder
	Alberta	1950	Chicken,			
			Canaries		+	Bigland
	Ontario		Chinchilla	+		Bain
			Cow, Sheep		+	
	Chesterfield, Morse Island (Arctic)		Brown lemmings		+	Plummer & Byrne
	Toronto (?)	1952	Lemming	+		Barrales
	Ontario	1953	Cattle		+	Barnum (317)
	Ontario	1953	Chinchilla, Rats			Avery (317)
	Alberta		Fowls			(317)
	Saskatchewan		Sheep, goats		+	Burton (317)
Ceylon	Kandy	1953	Goose	+		Bandaranayake
Denmark		1942	Lamb		+	Jepsen
Germany	Grenzmark, Neumark, Waertheland, Pomerania Saxony	1940-43	Sheep, Fowl	+	+	Pallaske

(continued on next page)

(Table 7 continued)

	East Prussia	1941/42	Sheep, Chicken		+	Pothmann
	Hesse	1943	Rabbits	+		Traub
	Hesse	1941-44	Duck, Goose Mouse, Horse		+	Veterinary school, Geissen
		1944	Foals		+	Krage
	Hesse	1946	Angora rabbits	+		Schoop
		1950/51	Pigs	+	+	Schoop
	Upper Hesse (Wetterau)	1936-39	Cattle	+		Beller & Zeller
		1949	Sheep		+	Beller
	Leipzig (zoo)	1950	Blue eagle		+	Schulze
	Frankfurt/am Main	1951	Cow		+	Özgen
	Saxony	1951	Cow (milk)		+	Potel
	Saxony	1952/53	Cow	+?		Dobberkau cited (323)
			Silver fox		+	cited (167)
	Lower Saxony		Sheep, Pig		+	Schulte (328)
	Mecklenberg	1952/53	Sheep		+	Veterinary research offie Greifswald (313)
	Rhineland (Bonn)	1955	Sheep		+	Scholz (pers.communic)
England	Cambridge	1923	Guinea pigs, Rabbits	+		Murray, Swann & Webb
		1937	Chickens, Turkeys	+		Paterson
		1938	Aborted lamb		+	Paterson
		1938/39	Chickens		+	Watkins (190)
	Cambridge	1939/40	Sheep, Goat, Hens, Rabbits		+	Paterson
	North Wales	1941	Calf		+	Harbour
Finnland		1952/53	Guinea pigs, Cow		+	Reine, et al. Stenberg, after (321)
France	Tours	1943	Sheep		+	Belin et al.
	Paris	1941	Fowls		+	Forgeot, et al.
	Tours	1946	Horse		+	Belin
		1952	Hare		+	Vallée
	Paris	1950-52	Sheep, Horses, Guinea pigs		+	Forgeot, et al. (cited 332)
Holland	Utrecht	1942	Pigs		+	DeBlieck & Jansen
	Utrecht	1943	Goat		+	Jansen and Van der Hark

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(Table 7 continued)

	Velawe	1947	Hen, Silver fox		+	Jansen & Peperkamp
	Friesland	1950/51	Canaries	+		Van der Schaaf and de Jong
	Friesland		Cattle		+	de Jong
	Friesland, Drenthe	1951	Cattle, aborted	+	+	Van Dorssen & Jansen
	Northern Holland	1951	Calf, Pig		+	Van Dorssen & Jansen
	Brabant, Utrecht, Overijssel					
	Overijssel, Drenthe, Gelderland	1951	Fowl		+	Van Dorssen & Jansen
	Friesland	1952	Aborted calves, pigs, hen, sheep		+	Van Ulsen (326)
India	Madras	1950	Sheep	+		Viswanathan
Israel		1951/52	Aborted calves		+	Levi, et al.
		1953	Aborted calves		+	Shamir
Japan	Sapporo	1948-51	Goat	+		Tajima
	Aomori prefect	1952	Goat, Sheep		+	Asabi
	Sapporo	1952	Sheep	+		Hirato, et al. see (119,245, 262,260)
New Zealand		1931-37	Sheep	+		Gill
Norway		1942	Sheep		+	Grini
		1943	Foals		+	Grini
		1948	Horse		+	Svenkerud
		1950	Sheep		+	Kieland, et al.
	Western Norway	1950				Naerland
		1951	Sheep	+		Svenkerud
		1951	Sheep		+	Odegaard, et al.
Poland	Posen, German	1940-43		+	+	Pallaske
Sweden		1910	Rabbits (?)		+	Hilphers
		1929	Dog		+	Hedström
		1942 up to 1943	Mountain cock			Lillengean
			Aborted calves, Lamb, Sheep, Rabbits, Hare, Dog, Blue- and Silver foxes, Fowl, Guinea pigs.		+	Wramby

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(Table 7 continued)

		1943	Hare		+	Hinrichson
		1945	Aborted sheep and calves		+	Olson (185)
		1949-53	Hens, Cocks, Roe, Hares, Fox, Cow, lamb, Vole, Cats		+	Thal (331)
Switzerland	Canton of Zurich	1955	Pigs		+	Schlegel & Frey (pub.note)
USSR	Ukraine	1939	Pigs	+		Slabospickij & Svincev
	White Russia	1942-45	Rabbit, Guinea pig, Mouse	+		Gudkowa and Sacharoff
		1953-54	Field mice	+		Alsufejew (335)
South Africa	Orange, Tiger River Distr.	1925-26	Jumping rodent	+		Pirie
Czecho-slovakia	Slovakia	1953			+	Patocka et al.
Turkey		1945	Pregnant mares	+		Ilhami Ozcebe (343)
USA	New Jersey	1931	Sheep		+	Ten Broeck (cited 240)
	New Jersey	1934	Cow		+	Jones & Little
	New Jersey	1934-36	Goat		+	Ten Broeck & King
	New Jersey	1935	Chickens	+		Seastone
	Connecticut	1937	Sheep		+	Jungherr
	Illinois	1938-40	Sheep, Cow, Bovine fetus	+	+	Graham and others (86a,b,d,f)
	Iowa	1939/40	Sheep, Pig		+	Biester & Schwarte
	Middle West	1939	Silver fox	+		Cromwell et al.
		1940	Horse		+	Jones (131a)
		1940	Fowls	+		Cole
	New York	1940	Sheep, Cow Goat (Rat)	+	+	Olafson
	Illinois	1941	Fowl		+	Hurt, et al (342b)
		1941	Sheep	+		Henderson
		1941	Sheep	+		Jensen & Gay
	California	1941/42	Sheep, Hen		+	Hoffmann
		1942	Bovine fetus		+	Evans & Sawyer

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(Table 7 continued)

Oregon	1942	Sheep	+		Muth & Morrill (345a)
Illinois	1943	Goat, Sheep	+		Graham, et al.
Illinois	1944	Fowl		+	Feisenfeld (68b)
	1944	Cow, Aborted sheep fetus			Poppensiek
S.-W. Virginia	1945	Cow		+	Hatch
Illinois	1945	Pig		+	Kerlin & Graham
	1945	Goat		+	Kaplan & Lager
	1945	Dog		+	Cox
Wisconsin	1944				Spencer, et al.
Michigan	1946-53	Sheep, Cow	+	+	Gray, et al. (87d-k)
Pennsylvania	1946	Cow		+	Boucher
	1946	Cow		+	Cole
Connecticut	1947	Wild raccoon		+	Gifford
	1947	Dog		+	Chapman
Ohio	1947	Sheep, Cow	+	+	Pounden, et al.
	1948	Sheep	+		Ryff & Lee
	1948	Pig	+		Rhodes, et al.
	1948	Arvicoline vole		+	Levy
	1949	Cow		+	Sellers, et al.
	1949	Cow, Sheep	+		Jensen & Mackey
Washington	1949	Chinchilla	+		Shalkop
Virginia	1949	Chinchilla	+		Shalkop
Utah	1949	Sheep	+		Stoenner, et al.
New Jersey	1949-50	Cow	+		Zink, et al.
	1950	Cow			Ward
Washington	1950	Ferret		+	Morris, et al.
	1951	Aborted cow		+	Ferguson
	1951	Pig, Hen		+	Bolin, et al.
	1951	Pig		+	Helmboldt, et al. (341a)
	1952	Cattle		+	Anderson
Nebraska	1951-53	Sheep	+		Olson, et al.
	1952	Pigs		+	Woodring
North Dakota	1952	Pigs		+	Eveleth, et al.
North Dakota	1943-53	Sheep, Cows	+		Eveleth, et al. (66a,b)

This long list (table 7) shows, therefore, the definite existence of listeriosis in 24 countries and almost 30 different kinds of animals and permits the impression of a widespread epi- and enzootic distribution of the disease in the animal kingdom. Characteristically--as for all less well investigated diseases-- the location of the sources are in the neighborhood of research institutions, universities, and so forth. Many findings, particularly among laboratory animals, were, according to the statements of the authors, purely chance discoveries, to which were appended systematic studies.

Also in Germany the sources of infection discovered to date are almost exclusively in the neighborhood of veterinary schools and a few veterinary research institutions; they are attached to such names as Pallaske, Traub, Beller, Schoop, and others. Vast areas of our hand are, however, not entirely unexplored. Occasionally a reference to human illness may thereupon, in regions where the disease is still not yet known to exist in animals, perhaps play a considerable role in determining the presence of the same.

2. Attacks among wild animals.

Although one of the first reports demonstrated the occurrence of listeriosis among wild animals, our knowledge of this sector today remains quite sketchy.

Pirie in 1925 found, during a control test for plague bacilli, in the Tiger River District and in the eastern part of the Orange Free State, (35 miles west of Johannesburg, 145 miles distant from the Tiger River Station) a carcass of a South African jumping rodent (Tatera lobengulae) which had died from typical symptoms of a septic pyemic Listeria infection.

He concluded from experimental studies on small rodents (ten kinds of mice, two kinds of rats) and larger rodents (hares, squirrels, rabbits) that pathogenicity was lacking for the larger rodents, and concluded the same for humans and larger animals. One might therefore attribute the annihilation of the jumping rodents to listeriosis-infected parasites. This experiment missed the mark (luckily perhaps), (281a).

Only once was the microorganism found in trapped rats in Northern Brazil (160). In connection with this it should be mentioned that the rat is supposed to be a natural reservoir for listeriosis, (184) for which today we have ample proof, of course. It is a matter of record that despite widespread mass surveys in the field of plague prophylaxis that L. monocytogenes is practically never isolated (281a) so that this leaves little possibility that the disease or ability to carry the microorganism is present to a great extent among rats.

If today a natural reservoir of the infection has not yet been proved definitely to be in existence, there are still basic

suspicions which lead to a search for it among rodents. These are indicated by findings among Lemmings that were trapped in the Canadian arctic. Since some of the animals (Lemmus trimucronatus trimuconatus and Lemmus groenlandicus groenlandicus) became ill of listeriosis only after transport and possible diminution of resistance, a latent capacity for carrying the microorganism may be postulated (197). Similar conclusions appear to apply to the vole also (153). Findings of listeriosis in mice are reported in batches from the Soviet Union, isolated instances are reported in Germany and Sweden (see table 7). The frequent finding of the presence of listeriosis among wild rabbits (92) and hares in the Soviet Union deserves attention. The latter were also in Sweden found to be infected with Listeria (169,331), where among the dying animals were found pregnant female hares carrying dead fetuses (293). Lately the disease has also been known to invade the French rabbit ranches (272).

The detection of cases of listeriosis among mountain cocks (155) as well as in foxes and roe in Sweden (331), indicates that this disease is more widespread among animals of the forest than is generally estimated. The single observation in the case of the wild raccoon in the USA (81) speaks out with the same significance.

The course of the disease and the pathologic anatomic picture corresponds largely to the phenomena in cases of experimental infection

and to the disease among laboratory animals. One finds in rodents widespread septic-pyemic forms with involvement of liver, spleen, heart muscle and lungs, in the case of birds, myocardial necrosis and liver granulomas, and in pregnant animals a purulent endometritis with septically infected and macerated fetuses.

Necrospy findings on a few wild animals showed: in roes subacute, fibrinopurulent pericarditis, acute splenitis, in the case of foxes and voles, liver necrosis (331).

Since the disease may also take a chronic, creeping, course, and as there exist healthy carriers of the microorganism, it is not out of the question that an occasional transmission from wild animals to human beings results. In bacteriologic examination of dead wild animals, it would be advisable to look for Listeria also. Probably they are hiding--just as in the case of human beings--under the so-called cases of "pseudotuberculosis" in addition to Pasteurella cases, also instances of listeriosis.

To what extent the wild animals are infected with Listeria, is not known.

3. Attacks among laboratory breeding and domestic animals.

a) Laboratory animals. Naturally listeria-infections are relatively seldom observed among the animal specimens of the laboratory. In consideration of the fine possibilities for study and the expert knowledge

of its validity, it is perhaps an essential point therefore, that under absolutely hygienic conditions of living and far reaching isolation of the individual animals outbreaks of the disease do not occur particularly if natural and high grade feed is at their disposal.

The only known instance of an extensive guinea pig epizootic appeared in 1923/24 in a laboratory at Cambridge. There it was discovered also as a widespread septic disease among rabbits (172). The pathologic-anatomic phenomena were described in the previous chapter. Distributed among rabbits are found cases with chiefly neurologic phenomena, that are expressed as lethargic states that are broken by intervals of convulsions, spasms, and cryings. Once Listeria were isolated from the heart blood of a guinea pig that had been infected with a virus strain at the Institute of Paris (232).

The first animal in which this disease was discovered at Cambridge was a pregnant rabbit. For the first time, 15 years later, Paterson (190f) was able to again observe the disease in Cambridge in an animal acquired elsewhere, and which died a few weeks after the mating.

The autopsy yielded liver necroses, purulent endometritis, local, adhesive peritonitis, and six dead fetuses.

It is remarkable that despite the greater susceptibility of mice no great epidemic in their cages has been discovered to date.

In 1950 in Washington successful detection was accomplished of L. monocytogenes from organs of ferrets that had been infected 10 days previously with a contagious virus causing distemper (169). The animals that were healthy up to the time of infection showed only distemper symptoms. How much of these were due to the virus and how much to the Listeria is not certain.

Also in certain kinds of lemmings (Dicrostonyx groenlandicus groenlandicus and Dicrostonyx groenlandicus richardsonii) listeriosis was found to appear; at the Toronto laboratories during trichinosis studies (16). In contrast to the septic nature of the naturally--occurring listeriosis in lemmings, in the arctic areas, the animals kept in the laboratory showed encephalitic phenomena.

As an isolated instance the findings of L. monocytogenes in the subcutaneous abscess of a rat (laboratory animal) in Canada should be mentioned (317).

b) Breeding animals. For the smaller breeding animals on fur farms listeriosis is a problem of economic importance that is not to be underestimated, if it slips into a non-infected herd and gets a foothold enzootically. It appears that for the disease to manifest itself clinically complex conditions must be fulfilled.

One such outbreak on an angora rabbit farm was reported lately by Traub. Three percent of the breeding animals took sick. In the

beginning only young animals were affected, later, also, valuable adult breeding rabbits. Clinically and pathologic-anatomically it was a question, regularly, of purulent meningoencephalitis with a remarkably protracted course. In no case did full recovery occur. Symptoms of its onset were not observed. All affected animals were free from fever. The chief symptom consisted of a permanent twisting of the head on the long axis of the body, in sidewise bending of the backbone (scoliosis) and finally in quick rolling motions along the long axis.

In connection with this it should be mentioned (231) that the same microorganism was isolated not only from the brains of seven angora rabbits but also from a healthy chicken.

The form taken by listeriosis most often in rodents--namely, sepsis, was prominent in an epizootic among a ranch of 500 angora rabbits of which 130 animals took sick and died within 14 days (231). All diseased animals were pregnant and had besides septic phenomena a purulent endometritis, pyometra, and local peritonitis. The fetuses were infected with the microorganisms; but it did not lead to abortion. The few surviving female hares were rendered unfit for breeding as a result of pyometra-, peri- and para-metritis.

Various outbreaks among chinchillas occurred in 1949 and 1950 causing moderately considerable loss of animals on Canadian and North

American farms that was likewise epizootic in character among ranches of silver foxes (Lit. Table 7). The latter showed clinically a distemper--like picture, a further proof for the fact that clinical diagnosis of distemper may not only hide a virus disease but also infections by protozoa (toxoplasmosis) and by bacteria (listeriosis).

Listeria-infections are commonly found in zoological gardens too (235).

c) Domestic Animals. Almost all domestic animals are susceptible to listeriosis; among them--if one limits oneself to the reports that have appeared to date--obviously, sheep and cattle are most often afflicted; after them follow goats, hogs and fowl. The horse may on occasion become ill also. Only a few cases have been found to date among dogs and cats. Even canary birds are not spared.

Listeriosis has considerable economic importance as an epizootic among cattle ranches.

If also in the literature many times only single instances have been repoted, one should not be deluded into thinking, concerning them, that since isolated observations by no means always indicate isolated cases, of disease, because the disease often taken an atypical course, consequently only the fatal cases come under investigation. Time and again one finds statements to the effect that even before the etiologic determination of a fatal case, other animals have died, in the meantime, from similar symptoms.

Sheep. The presence of extensive listeriosis was discovered for the first time at the beginning of the (19)30's in sheep ranches of New Zealand and Western Australia (82) where the plague because of its clinical phenomena passed under the name of circling disease, a designation that was also current among the East German sheepfolds. These reports included numerous further observations, first in the USA, later in Germany, England and other countries in which sheep breeding thrives, whereby it could generally be noted that, to date, pertinent reports are still to come from the sheep breeding areas of southern Europe, the Asiatic border lands, and South America. In the last year the disease has been discovered in sheep in India and Japan also.

Listeriosis outbreaks among sheep are often very costly in losses.

Biester and Schwarte report, for example, that of 225 young animals that took sick among a range of 2200 sheep only 3 lambs survived the outbreak of the plague. Pallaske figured the fatality at 15-20 percent.

Among older animals the course of the disease--as already explained on page 36 (of the text)--manifests itself mostly as encephalitis, encephalomyelitis, or meningoencephalitis (29,82,86a,f, 87d,g, 134,186,189,206 and others).

This course of disease was only occasionally found among lambs (87f,g,h).

In the foreground of the clinical symptoms stands, in the case of young animals, chiefly a septic disease picture, that is characterized by widespread liver damage (see Figures 14 and 15).

Because other diseases with similar symptoms may exist concurrently, the diagnosis of listeriosis in sheep is not to be made from the clinical symptomatology, just as in bovine listeriosis that will be discussed later.

Confusion with hemorrhagic septicemias are quite possible. American authors (86f, 87g) report that ketoses, enterotoxemias, and overeating may hardly be differentiated clinically from Listeria-infection and an etiologic diagnosis in common outbreaks of these diseases in a herd is first successful in practice only on the dissecting table. In listeriosis in sheep the fatty liver characteristic for ketosis is absent and likewise the spreading subserous hemorrhages of the intestinal tract and peritoneum. Considerable difficulty is encountered in the differentiation of encephalitis due to viruses, louping ill, from avitaminoses, and also from the circling disease (189) caused by Coenurus cerebralis. Bacteriologic procedures are indispensable for diagnosis. How far therein a partnership between listeriosis and a virus infection play a role, future studies must show.

The clinical symptoms generally begin with an increase in temperature and increase in water and food intake. These are believed many times to indicate more or less widespread neurologic injuries; teeth grinding, jaw muscle stiffness, stiff gait; the animal moves in circles with its head bent sideways; (riding school movements) of which the range is limited by the bending that is present, and it tries to rest against fences, on feeding troughs and so on. Runny noses, conjunctivitis, and keratitis often appear. In the far advanced stages limitations to motion are manifest with crippling of the hind- and fore-foot; it reaches stages where it resembles uterine paralysis of cattle. The animals are no longer able to lift themselves and finally die. Hematologically one finds, as a rule, a definite leukocytosis with concomitant lymphopenia (186a).

In sheep listeriosis is recognized as having a quite stormy course; often death results only 2-3 days after appearance of the first clinical symptom. Occassionally the disease lasts 7 to 10 days.

Lately mild courses of the disease are recognized which are poor in symptoms or are symptom free and exhibit no symptoms besides increase in temperature and of leukocytes (186b).

Permanent damage develops in healed cases, for example, torticollis, which are considered the result of encephalitis (186b).

Figure 17. Young sheep with listeriosis, that is leaning against a fixed object. Observe the mucopurulent exudate from the nose, outthrust tongue, and the snow on its wool (an indication that it cannot maintain its equilibrium). (By permission of the J. Am. Vet. M.A., after a work of Gray and coworkers, ibid. 118: 242, 1951)

The nose is supposed to be the port of entry for the microorganism (82,189). Experimental studies to determine the rhinogenous transmission of listeriosis were successful in instillation of suspensions onto the nasal mucosa and after previous scarification of the mucosa (7,82) but missed the mark in other instances. On the whole, it has not been possible to date in practice, to cause listeriosis in sheep under biologic conditions. Thus, one still knows little about its pathogenesis. But after Listeria were found in the noses of healthy sheep (245) the hypothesis of Gill, Pallaske, and others may yet be right.

Definite reports on the incubation time do not exist. It amounts plainly to up to 3 weeks (87g).

Apparently the epizootic outbreak of the disease is determined by the seasons. Pallaske and Gray and others report from their own observations of this, that the overwhelming majority of the cases of disease occur in winter and the beginning of spring. This is true only to a limited extent, since outbreaks have been shown to occur in considerable numbers in the summer also (251).

Listeriosis deserves special attention as the cause of infectious premature lambing (190e). The pregnant animal becomes ill mainly from

a purulent metritis, generally without attendant phenomena on the part of the liver or the CNS and does not itself die from the infection. Septic infection of the fetuses results from intrauterine displacental transmission with the result being abortion and still-birth. If infection results first near the end of pregnancy, oftener still-living animals will be born, but these mostly die within a short time of septic listeriosis. The organism has been successfully isolated from the organs of the fetuses (for example, from the fourth stomach) and also from the afterbirth.

Figure 18. A month old lamb with listeriosis. (Torticollis, dilated pupils, and strabismus) (Gray)

A larger outbreak, during the course of which 16 animals aborted, gave rise to experimental studies among pregnant sheep. After repeated peroral intake of large quantities of bacteria three mother animals became lame on the right side and brought healthy animals into the world. On the contrary, all animals aborted on the 7th to 12th day after intravenous infection, equally so, whether they were only 2 1/2 months pregnant or were almost at full term. Only one animal gave birth to living twins both of which however died shortly thereafter of septic listeriosis (190c).

Paterson (190c) has already called attention to the obvious parallel between intrauterine-induced listeriosis of young sheep and

similar cases reported in humans. Almost 15 years later Potel and his coworkers were able to prove this hypothesis conclusively in perhaps the most important course of human listeriosis.

Not always however does the infection of the mother animal result in crippling, as another outbreak in a herd of 150 animals with 19 fatal cases teaches (186a):

In many cases premature birth resulted without subsequent illness. Eight fetuses that were found in the uterus of animals that died of listeriosis, were free of Listeria, as was also the placenta. No abortion occurred during the entire epizootic. -- On the contrary all pregnant sheep aborted between the 7th and 11th day after artificial infection and brought dead or very sick lambs into the world.

To sup up, it is thus shown, that all appearances of listeriosis in sheep may run their course under different symptom complexes. The isolated disease of the brain stem is indeed the most frequent course form. In this kind of cases, passage of the bacteria through the placenta doesnot appear to occur (186e).

Goats. Since listeriosis in goats has no important differences from that disease in sheep, special discussion is not needed. Asymptomatic infections may occur (127a).

Pigs. Pigs, also, are similarly attacked by this disease that in many countries--including Germany--is relatively frequent. Listeriosis has been observed at various times (143,212) in combination with hog

cholera. By conjunctival infection an encephalitis analogous to the natural picture of the disease was successfully aroused (86). It is a question principally of a disease of young animals (pig-grippe?) that now and then also occurs in weanlings and larger piglets. Clinically, tremors, spastic paralyses, coordination damage, and convulsions are prominent. Death often occurs after only 48 hours--. Occasionally Listeria detection is successful also as a chance finding in other diseases, for example, in alkali poisoning (35a).

Cattle. Bovine listeriosis deserves a thorough consideration, as it has attained a considerable significance as an epizootic in cattle ranches of North America. Juliannelle has stated a long time ago that listeriosis was in its importance perhaps the equal of brucellosis. Today it appears that--at least in some areas--it already causes greater damage than Bang's disease. In all encephalitic processes, for differential diagnostic purposes, a bovine listeriosis must be considered. Confusion with rabies and/or poisoning are repeatedly reported (86a,b,f, 87g).

Some disease symptoms are so characteristic, that they may be viewed as leading symptoms of bovine listeriosis.

At the beginning of the illness the animal separates itself from the common herd, looks for a corner, or leans against the fences or other fixed objects. According to observations it generally then

begins to move in circles. The general body state is obviously severely affected and food will be refused. Generally the temperature rises. In some animals a conjunctivitis develops in both eyes. Noteworthy are the profuse secretion from the nose and the exudation of viscous saliva. The head is generally sunken to one side, and from which the ear hangs limply. The tongue protrudes from the mouth. According to published studies they show damage to sight and mobility, besides increasing emaciation and usually a more or less outspoken irritability, that may lead to confusion with rabies (86,87).

In the last stage the animal is comatose. It can still survive for one or two days in this state, until it finally dies.

In contrast to the acute course of the disease in sheep, the duration of the disease in cattle comprises an average of two weeks after the appearance of the first symptom. Some American reports indicate a mortality rate of nearly 100 percent. However, there are also a number of spontaneous cures reported (29,86,87,128,186, and others). Cases with a hyperacute course seldom survive (207a).

During the course of disease a leukocytosis as well as a leukopenia may be found in blood count observations. According to Graham, Hester and Levine these apparent contradictions depend upon the circumstance, that the blood was taken for examination during different stages of the disease. After experimental infection generally

results an initial leukocytosis and subsequent leukopenia, that, can, however, fail to occur. The increase in monocytes in ruminants is not pathognomonic for listeriosis. Also in cows the connection between listeriosis and premature calving was demonstrated (69,86b, 152,185,258,277,293).

One may successfully induce abortion in about ten days after artificial intravenous infection of a cow that is 5 months gravid, whereby a brucellosis may with certainty be excluded. During the search for bacteria in the organs of the septically infected fetus, which was successful, the placenta, blood, and colostrum of the mother animal were found free of Listeria. The research animal quickly recovered and showed, in the convalescent stage, a rise in Listeria titer of from 1:50 (doubtful) to 1:6400 and later to 1:50,000 (86b).

Also under natural conditions most of the mother animals, obviously, survive the disease, which runs its course under the picture of a uterine catarrh. In this kind of cases Listeria can be isolated from the slimy exudate (256)and it is completely possible that these bacteria may be more frequently found in the genitals than is acknowledged today (321) perhaps even as normal inhabitants thereof (69,87m,185). In any case there are unmistakeable parallels to the premature lambing induced by listeriosis. Nevertheless, it is still not known how extensive a part listeriosis plays in infectious premature calving.

It should not be left unmentioned that in young cows listeriosis may appear with the features of milk fever or uterine paralysis (72).

The morbidity in listeriosis—encephalitis is distinguishable in afflicted herds. According to American authors as a rule not more than 15-20 percent of the animals are attacked. This does not exclude (the possibility) that the greater part of the herd has a latent infection, or experiences atypical forms of listeriosis. Among a herd of 27 cows, for example, 8 may die of listeriosis-encephalitis, while the remainder have only a mild conjunctivitis.

The labors of German scientists have provided some interesting contributions to the question of atypical bovine listeriosis. Part of the observations go back to 1936 and are concerned chiefly with the etiology of virulent, infectious catarrhal fevers, that according to Ernst should be calledthe cattle form of Borna's disease (cited in 22).

Figure 19. Cow ill with listeriosis (Note position of head, drooping right ear, pretruding tongue, copious salivation). (By permission of the J. Am. Vet. M.A. after Gray and coworkers, ibid. 118: 242, 1951)

This view has not, however, been generally accepted. First the endeavors of Witte, and later the widespread studies of Beller and his coworkers are to be thanked that a far reaching explanation

was finally developed. All cases that manifested themselves clinically in the form of severe catarrhal symptoms in the croup membranes, the nasal mucosa, in the trachea or bronchi, or as lobar or lobular pneumonias, had this in common, that the bacteriologic detection of L. monocytogenes succeeded. The bacteria were immediately designated as "Vilbeler bacilli". In a series of cases the Borna-virus was morever, found, a finding that should not be underestimated. Whether it is here a question of a chance association or of a conformity to a law, may be clarified by further research. In any case, Listeria must be considered as an essential factor for the appearance of virulent catarrhal fevers (22).

Listeriosis appears to play a role, furthermore, in the genesis of mastitis, although at the present time a less important one.

Nyfeldt and Schmidt, in 1938, observed apropos of a small epidemic of human listeriosis, that this apparently resulted from infected milk. Wramby later cultured Listeria from milk and from the udder of animals ill with mastitis, and in 1951 Potel and coworkers,--independently of the authors--named investigated the significance of infected milk and were able to culture the bacteria from one such case. The studies conducted by Halle (61,204f,323) in this sphere yielded hypotheses for that, that atypical mastitis was probably due to Listeria also. A certain reserve is now appropriate in the explanation of these findings obtained exclusively by serology, since one knows relatively little about the significance of the Listeria titers.

Reports on the titer values in animals and their significance are to be found summarized in the chapter on the serodiagnosis of listeriosis (p. 118 ff).

In a few animals that survive listeriosis of the CNS, typical postencephalitic or postmyelitic sequelae appear. Such animals appear dumb, have difficulty in seeking food, fall frequently, and cannot hold their own in a herd. Similar phenomena are also seen in sheep.

Gray and Moore studied brains and medullas of six cows that had survived an outbreak of listeriosis in a herd for one year before being slaughtered, and before they were themselves taken sick. Listeria detection by culture was no longer successful. Tissue sections revealed well defined areas of connective tissue that became more heavily stained than the surrounding tissues. In a few foci of this kind they found a few lymphocytes, others consisting of hyaline material, and a substance which they concluded was formed by calcium deposit. To all appearances it was a question, in connective-tissue, of healed foci of listeriosis.

Horses. A few observations exist of listeriosis in horses (21,89,149,261, and others). Encephalitic symptoms predominate, the diseased animals show increased irritability, esophageal paralysis, and concomitant stomatitis and inflammation of the masseters, with

resultant difficulty in nutritional intake (feeding), besides a not seldom presence of purulent keratoconjunctivitis. -- Death occurs generally about ten days after the appearance of the first symptoms of the disease. -- Many times Listeria will be discovered in cases of clinical Borna's disease and as a source of septic pyemia of the foals. -- In a extensive outbreak in a herd of 200 pregnant mares, that occured in 1945 in Turkey, 12 animals had symptoms of an encephalitis and died within about 5 days. Premature foaling resulted neither in the diseased animals nor in the ones that remained healthy (343).

Fowls. In fowls listeriosis appear sporadically and epizootically as an acute or chronic sepsis. Liver and heart muscle involvement increase in frequency. The heart muscle is covered with pinpoint-like, gray-white, swollen foci, similar to those in the case of pullorum infection. Besides the myocarditis there often develops an epicarditis with fibrinous deposits and an adhesive pericarditis as well. The pericardium is then filled with an amber liquid. Healthy carriers of bacteria are found many times. The microorganism may also be isolated in the course of leukemic disease of fowls, according to the other reports, from the liver that is not altered pathologic-anatomically, of animals that died suddenly, also from the spleen in a clinical Coryza (342a).

Sick chickens often begin to go lame, to become thin, and to go blind (209). Often one finds isolated ovarian inflammation as the only indication of listeriosis. Concomitant appearances of asymptomatic listeriosis with Newcastle-disease have been reported in the U.S.A. (35b). -- In canaries the disease runs a predominantly septic course (30,275) the same as in turkeys, ducks and geese, in which in Ceylon neurologic damage was also discovered (14).

Dogs. Dogs are also susceptible to listeriosis (43). Clinically the disease ran, in a six months old dog, like the course of a distemper. Central nervous system damage and fading sight were the most important symptoms. In the differential blood count a definite monocytosis was detected.

Since the pathologic-anatomical findings have already been described above (p. 32 ff) with respect to their salient characteristics, a few more descriptions pertaining particularly to this kind of animal, may be listed here. Individual reports may be followed up in the literature cited.

A peculiarity that one finds more often in listeriosis under natural conditions than in experimental studies consists of the observation that during the course of this disease, considerable pericardial, pleural, and peritoneal effusions may arise. The exudate curdles remarkably swiftly in a reagent glass

and contains a large quantity of triphosphate crystals.

In conclusion it is to be emphasized that it is certain that still other disease syndromes exist in which listeriosis plays a causative role. This is especially true of all domestic animals in which with considerable frequency arises a detectable honeycombed liver necrosis. Whether it is here a question of a special form of listeriosis, is still not certainly determined. After all, the bacteriologic findings would lead one to just such a suspicion (Rubarth and Wollatz).

There is little to be said about pathogenesis, as hardly any definite reports exist as to the point of entry and the dissemination of the microorganism in the body of the animal under natural conditions. The results of experimental studies referred to above vary considerably.

Of considerable practical significance may be the answering of the question as to why, in infected herds, generally only a few animals fall ill with myelitis and encephalitis. Additional factors in the form of a listeriosis-enhancing agent (Olson 321) among which may possibly be concealed one or more viruses that, in any case appear to influence the course of the disease considerably.

4. Epizootiology.

Notwithstanding the large number of casuistic and methodical

contributions to the theme of listeriosis in animals our understanding of the epizootiologic provisions remains to date quite full of gaps. A natural reservoir of the microorganism is not known, although at the same time it has been supposed many times that rodents--particularly rats--play a role in this. The existence of healthy carriers of the microorganism has been reported among wild rats of Northern Brazil (160). Olafson (184) reports, that over a period of many years, the time of greatest frequency of listeriosis cases among caged animals corresponded to the time of greatest abundance of rats i.e., during the winter months.

It is commonly supposed that a living carrier is to be held responsible for the dissemination. Gill points out that Oestrus ovis, a kind of fly often found living in the noses of sheep, may be involved in the transmission of this disease from one animal to the other. However, there is no proof of this kind of a relationship.

Whether the variety of mite Dermanyssus gallinae found in infected canaries or other kinds of mites in the case of fowls are involved in the spread of listeriosis cannot yet be decided.

The formulation of chains of infection in the sense of Gotschlich (93) is wrecked upon the ignorance of the mode of transmission and of the point of entry of the microorganism. Outbreaks occur, for example, in neighboring herds without there

having been any known direct contact between them (87g). Of course, it could have been imported into both herds from a foreign source. This gives rise to the hypothesis that among the larger animals themselves exist latent, infected, carriers of bacilli, that infect uninfiltrated herds in a manner as yet unknown. A series of cases of listeriosis have occurred after the transportation of large and small animals for considerable distances.

This points to outside influences being extensively involved in the development of the disease, for example, changes in environment and changes in conditions of feeding, and further, however, also the yearly variations and meteorotropic influences. As already explained, it often but not always happens that the maximum mortality in listeriosis occurs during the winter months and the beginning of spring, thus, at a time, when fresh fodder is no longer available. Special attention has been devoted to the question as to whether infected fodder is involved in the genesis of listeriosis.

Gray and coworkers found for example in researchon artificially infected rabbit and cattle fodder, hay, straw, and wood shavings, that Listeria survived for 6 to 26 weeks, but could not produce any criterion thereby, that under natural conditions straw, hay and feed, and soom, or the drinking water in infected hutches, any any influence on the dissemination of the infection. They considered it unlikely

that much could be learned about the details of the epizootiology as long as the biologic reservoir of the infection has not been found.

The possibility of transmission to young animals by means of infected milk is also of increased importance in the reckoning.

The question is repeatedly brought up as to whether silo fodder could play a role in the development of the disease, immediately after a connection was postulated showing that a definite rise in listeriosis morbidity often went along with the feeding of grain from the silo. (Jensen cited accordingly 186c (86f,128,184)). In spite of the study of Olson and coworkers upon this point, there is today no lucidity on it.

Outbreaks do not as a rule occur explosively. Indeed, many animals sicken at the same time, but often weeks and months pass between the appearance of clinically manifest cases of the disease. This fact applies usually only to the generalized encephalitic form of listeriosis in larger animals. The proportion of infections among 7,000 sheep, for example, amounted to an average of 8 to 9 percent (128), in individual herds between 3.3 and 15 percent; among 85,000 cattle the frequency of infection varied between 0.03 and 7.5 percent. Probably however only a small percentage of the animals with widespread involvement of the central nervous system became ill while instances of atypical courses of the disease (mastitis,

conjunctivitis, etc.) stood our prominently. Since these typical disease forms have been given relatively little attention to date, it is a widely held view that these epizootics of listeriosis run a slow, circuitous course. The observations in the middle of Germany speak out against this, in that listeriosis can run through cattle herds as an atypical mastitis with relative swiftness (204f). As to just what extent the contact between the animals or perhaps also transmission by the hands of the milker may play a role, that question must remain open. The observation of Gray and coworkers than in an infected herd only those animals became sick that had had contact with one another shows, anyhow, that contact infection is epizootically important in the case of listeriosis.

Not to be underestimated also is the association of Listeria- and virus-infections. It was noted only in the repots on the virulent catarrhal fever of cattle or the Borna's disease among other large animals. Whether the single chance observation of the Borna virus in the case of catarrhal fever is the result of an accidental finding that is of no significance for the course of the disease, or there is a relationship here, must be explained by future research.

Diminution of resistance for various reasons has a definite influence on the course of the disease and on the pathogenesis in individual cases.

A series of observations indicates that the strain may be transmitted from sheep to cattle herds.

For example, cattle became ill in a pasture that had formerly been used for sheep. The outbreak described by Beller and Zeller in the Wetterau in 1936 contains valuable information on this. Although no sheep were held in the stricken area itself, at the aforementioned time an itinerant shepherd from Thuringia, (where listeriosis is present among sheep) grazed his flock along a meadow in the vicinity of the road. In the future only those animals sickened in the double rowed stall that had grazed upon the meadow and indeed regularly even then when the east wind blew. The author concluded that one must suspect a transmission of the infectious agent by the airways.

On the other side Gray, and others saw that cows remained free from clinical listeriosis, although they were kept in the same stall with infected sheep.

The hypothesis of an aerogenous route of transmission (22) was supported by the findings in the epidemiology of human listeriosis (see page 93 of text).

In the case of the Listeria it is a question as to whether bacteria, which remain alive for along time in dirt, and also in dust, retain their ability to cause infection. Just as in the case of the Norwegian farmer who was himself infected by dust containing micro-

organisms when he was cleaning his stable (page 93) is also in other cases the possibility to be reckoned with, that the spread of listeriosis in animals results from the distribution by way of infected dust and dirt.

It is certain that this is only one of many ways of becoming infected. A series of authors (7,82,189) report on a rhinogenic origin, wherefore the results of a few experimental studies and later observations (245) are in agreement. Traub reports on an otogenic transmission method, while Schulz believes the peroral method the most likely. Parenteral infection should only exceptionally come into consideration.

Little attention has been given to date to the possibility of infection while covering. Gray (308) made here an important observation, that he was once successful in culturing Listeria from the urethra of a bull. Thus it is not impossible that a herd could in this way become infected. How the intravaginal infection can be detected clinically and whether this state of things influences abortion must still be investigated (255).

The only definitely certain biologic pathway of infection takes place diaplacentarily--intrauterine and results in septic infection of the fetuses following a purulent metritis of the mother animal. If this phenomenon occurs only near the end of

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The only definitely certain biologic pathway of infection takes place diaplacentarily--intrauterine and results in septic infection of the fetuses following a purulent metritis of the mother animal. If this phenomenon occurs only near the end of

a pregnancy, animals will come into the world that will mostly die within a short space of time from septic listeriosis. As a rule however intrauterine infection leads to the death of the fetuses and abortion. Probably a few animals may be infected intra partum (during birth), for example, from aspiration of the amniotic fluid containing Listeria.

The extraordinary susceptibility of all young animals to Listeria infection and the relative resistance of the older animals may be explained on the basis of the immaturity of the younger organisms, on the other hand, however, also by a latent infection and a passive immunization. The appearance of agglutinizing antibodies in sera of many large and small animals may then be easily determined.

But the bacteriologic--serologic type diagnosis of the Listeria will show that outbreaks among an individual herd generally originate from the same type, therefore one is dealing with a single or unique source of infection. For a long time the meaning was sought of the supposed strong connection between serologic types and their presence in rodents or ruminants. In fact, in listeriosis among rodents one finds mostly Paterson's Type I and in ruminants more often one finds Type 4. But Paterson (1940) and lately Seelinger (1951-1954) were able to demonstrate in the seroanalysis of 61 or

163 strains from all parts of the world (see table 8 below) that this was no hard and fast rule, but that the socalled rodent type was likewise present in sheep, cattle and goats and vice versa.

Meanwhile it was evident from the same type determinations of strains of diverse origin, that a direct relationship between listeriosis of rodents and ruminants exists only as a general range of possibility where serologic type identity existed.

A significant role in the epizootiology of ruminant listeriosis is perhaps played by fowls since here type 1 as well as type 4 exist in considerable frequency, and repetition of the same type has been observed among fowl, cattle and rodents from the same farm. The presence of melezitose-fermenting and--nonfermenting strains among the individual serotypes represents an increase of the possibilities of divisions into types but has nothing to do with the connection with definite habitats. In order to pass final judgement on this, study of a much larger number of strains is necessary. Findings of the authors (see table 5) speak, moreover, against a relationship between the habitat and the sero- and bio-types.

It should not be forgotten that despite far-reaching biochemical and serologic similarities an adaptation of many strain occurs in certain kinds of animals--analogous to the

Table 8. Type divisions of L. monocytogenes in humans and animals among 163 different strains available.

Serotype	Host										
	Human	Rodent	Fowl	Cattle	Horse	Sheep	Pigs	Goats	Roe	Ferret	Canary bird
1	60 USA, Australia, England, Germany, Canada, France, Austria.	12 England, South Africa, Canada, Arctic, Sweden, France.	13 England, Canada, Germany, Sweden, France.	5 Germany, Israel, Sweden.	1 Germany	5 USA, Sweden, Germany, France.	--	1 Japan	1 Sweden	--	1 Canada
2	1 Scotland										
3	6 Denmark					1 Germany					
4	27 USA, Argentina, Germany, Canada, England, France.	1 France	2 USA	7 USA, Canada, Israel.	1 France	12 USA, Japan, New Zealand.	1 USA	4 USA Japan	--	1 USA	--
Totals	94	13	15	12	2	18	1	5	1	1	1

Pasteurella--so that all in all the investigation of infection among the species from which the bacilli have been isolated should be the most successful procedure (189). These findings may however yield no generally valid conclusions, according to experimental studies, for example of Schuls and others, which yielded different results.

One of the chief obstacles to obtaining further insight into the epidemiology of listeriosis is contained in the farreaching cultural and biochemical similarity of the small number of easily differentiable serotypes and in the manifold pathogenetic potency of the microorganism. A series of unknown factors must certainly play a role in this, the determining of which will first bring light into the darkness, which surrounds the epizootiology of listeriosis.

5. Therapy and prophylaxis.

Up to 1939 there was no therapy for listeriosis. Porter and Hale were indeed the first to determine experimentally to what extent Listeria infection is susceptible to sulfonamides (S.A.), sulfanilamide and sulfapyridine.

They found under test conditions in experimental studies with white mice, good action by both drugs. This was confirmed by Webb and expanded in the process to show that by combined administration

of immune serum and oral chemotherapy with sulfapyridine a survival quota of 100 percent could be obtained in mice that had been infected with a dose 200 times higher than the fatal dose, and from 27 to 73 percent survival, under the same conditions with the same dose, when sulfa-therapy was used exclusively.

Therapeutic results were attained in sheep with large doses of prontosil (Frost and Danks, cit. (184)) as well as in cows with sulfanilamide. In contrast stands the failure of sulfonamides in listeriosis in a large herd of sheep (107).

The most promising is early treatment which led to healing in two cases so treated with sulfonamide and neoprontosil (129) and thus are to be recommended therapeutically as well as prophylactically (21,231).

There is no serum therapy against listeriosis, although protective substances appear in the serum of animals after surviving an infection (186b).

With the introduction of antibiotics in the therapy of infectious diseases the possibility of treating listeriosis was also enhanced. Through widespread research in vitro in the years 1945-1953 was determined the susceptibility of numerous Listeria cultures to the more important antibiotics. The findings are compared in a review of Linzenmeier and Seeliger with the results of their own tests and are discussed fully

in the chapter on the therapy of human listeriosis (157b).

On the basis of the differences in activity as determined in vitro, the tetracyclines (Aureomycin, Terramycin, Achromycin, Tetracyn) appear to have the best prospects for the antibiotic therapy of listeriosis. Then followed sulfonamide preparations and sulfa supplement products, for example Sapronal, the latter at best in connection with penicillin, that alone generally fails to work in practice. Resistance against streptomycin is rapidly developed (84). The use of chloramphenicol (chloromycetin) and lately of magnamycin (or of erythromycin) appears auspicious.

Results of in vivo studies stand in good correlation with the results, that were determined in vitro, of the susceptibility of Listeria against antibiotics and chemotherapeutic agents.

In studies on Listeria-infected mice, the administration of 500 Oxford units of penicillin had only a prophylacticaction if the drug was given immediately after infection. Already 2 hours later it was still only slightly capable of prolonging life (188a,c).

By studies on rabbits it was ascertained that streptomycin is contraindicated because of the quick development of resistance to it (87a).

In research with mice it was on the contrary successful (178) in achieving 100% survival after doses of germs that were fatal to

98 percent of the control animals by courses of aureomycin and 30 percent by courses of chloromycetin if the drug was administered immediately after infection. After a 24-hour interval between infection and injection of this drug even still 90 or 35 percent of the animals could be cured. 5 mg. aureomycin per kg. body weight made it possible also to protect 100% of intravenously infected rabbits and mice (298) if the medication was commenced 30 minutes after the infection. Penicillin remained without action under the same conditions (10 mg/kg.). Gray, Laine and Thorp showed additional favorable results in that, with aureomycin, 3 of 4 intravenously infected and 12 of 21 intracerebrally infected rabbits survived although the medication was, in nine animals, first administered 12 hours after infection (five animals died).

Local treatment of artificially infected rabbit eyes with an aureomycin ointment prevented the formation of a conjunctivitis in two of four animals and shortened the duration of conjunctival inflammation phenomena. The bacteria disappeared, however, from the treated and the untreated eyes after nearly the same number of days, a finding that may be traced back to only a bacteriostatic activity of aureomycin (87 1). The hope arising from results of experimental research for an antibiotic treatment for listeriosis in animals have been, however, only partially fulfilled.

Healing of bovine and ovine listeriosis, was, to be sure, reported after the administration of penicillin, but no others could be healed by this (128); in the case of listeriosis in dogs penicillin, in any case, failed to work (43).

Zink, de Mello and Burkhardt reported on the first experiences with aureomycin in 1951. It concerned a herd of cattle infected with listeriosis. In December 1949 2 sick cows were administered sulfanilamid (dosage not given) and each was given an intramuscular injection of 2 million Oxford units of penicillin in oil and they were both cured. Since 2 other animals with listeriosis encephalitis in the same herd, however, healed spontaneously, it is not certain whether the therapy alone is to be held responsible for the favorable outcome, since a fifth animal died a few months later despite combined sulfanilamide-penicillin therapy. In the case of a cow that took sick shortly thereafter, aureomycin was administered intravenously at the rate of 5 gm. on each of 3 consecutive days, without their being able to save the already moribund animal. The authors concluded that (the quantity administered was too small and so they had not been able to attain the concentrations of the drug in the brain that were necessary for arresting the disease.

Gray and Mooretreated 25 cows in a herd infected with listeriosis with aureomycin, (dose: 2.5 gm. of aureomycin hydrochloride, dissolved in 100 parts of sterile distilled water per day in the case of animals

under 600 American pounds in weight, 5.0 gm. for heavier animals) whereupon 14 animals survived. This quota was considerably higher than that which could have occurred from spontaneous healing. Some animals had only mild symptoms at the beginning of therapy (conjunctivitis) while others were already semimoribund. Two of these severely ill animals were, on the fourth day after the medication, which was administered during a period totalling 4 days, fully symptom-free. In all animals a definite lowering of temperature occurred within 12 hours. Since the etiologic diagnosis of listeriosis is not possible during the lifetime of an animal, the treatment was therefore based on clinical indications, that were, however, confirmed in the case of six animals that died, both bacteriologically and at autopsy.

The relatively favorable results in the treatment of bovine listeriosis is in contrast to the failure of aureomycin therapy in the case of ovine listeriosis. Of 13 sheep treated (dosage 5 mg. per American pound of body weight per day) only two survived the disease.

The American authors (87j) brought these striking differences into focus by the fact that listeriosis in cattle generally has a longer, more subacute course than in sheep, so that the drug has time to act. Sheep with acute listeriosis did not even survive, as a rule,

the 4-day treatment period. Histological and bacteriological study of sheep brains showed that considerable irreparable damage had already occurred at a time when there were still no clinical indications. On the basis of these findings it was concluded that in ovine listeriosis any drug therapy must be useless if definite clinical symptoms are already present.

This failure does not result from the inability of aureomycin to pass the blood-serum-barrier (87i,j). During the 4 hours after intravenous injection the drug was conveyed to the brain and medulla, to be sure, however, only in concentrations that did not prevent a subsequent spread of the bacteria into the brain substance.

For the therapy of listeriosis by means of antibiotics with broader spectrums of activity, the same criteria apply as for the use of sulfonamides: the earliest possible beginning of therapy and the highest possible doses. In far advanced cases however, only a slight influence is to be had in the case of drug therapy and at best will lead cases to an incomplete cure.

In the face of this situation that is still further complicated by the difficulty of making a purely clinical diagnosis, it is understandable that even as early as this prophylactic measures have been taken under consideration. Such a procedure runs up against the lack of knowledge of the pathogenesis and the epizootiology.

Studies of vaccination with Listeria-vaccines were made in this connection.

Numerous immunizing studies with chemically or physically killed Listeria in mice remained however without results. Julianelle ascertained in such studies that agglutinating antibodies did indeed appear in the serum but that these gave no protection against subsequent artificial infection. Graham, Levine and Morrill (86c,f,g) sought in vain by means of single or repeated subcutaneous injections of a formolized Listeria-suspension to immunize rabbits, guinea pigs, chickens and sheep against an artificial Listeria-infection. Özgen got the same results.

Also the course of the disease in mice was not influenced by the simultaneous administration of homologous antiserum with intraperitoneal infection (132a,b). Injection of bovine listeriosis antiserum of a titer of 1:50,000 was not able to passively immunize rabbits and guinea pigs (86c).

Graham and coworkers could induce no immunity in chickens and rabbits after subcutaneous injection with living bacterial suspensions. But Olson writes that two or three injections of living Listeria over a period of many weeks were enough to protect sheep against subsequent intraarterial infection with living bacteria (186a). In other studies of a similar nature eight of 20 intracerebrally infected animals

survived the disease, and six did not become ill at all (184).

These results which were not in themselves convincing gave impetus to the investigation of the value of active immunization under natural conditions. According to Olafson, vaccination with Listeria vaccines did not influence the course of the disease in a herd of sheep. Graham and Levine likewise got no better results (86g), although they administered large doses of formolized suspensions of Listeria. In a herd of sheep of about 1000 animals, in which during the winter months of 1938/1939 and 1940/1941 listeriosis was present with a fatality rate of 5-7 percent, a greater part of the animals were vaccinated once, some repeatedly. Thereby one or two injections each consisting of 20 ml. of the formol-vaccine had no protective action. Three injections spaced one week apart each, first led to the development of a relatively weak immunity, so that this dosage must be viewed as yet insufficient. An attempt to immunize sheep with living vaccine during an epizootic failed absolutely (186b).

Although the immunization studies of Eveleth cited above (86b) on 20 herds of sheep with over 5000 animals led to results similar in many respects to the foregoing, a definite protective action was, however, unmistakable. After an initial negative phase immediately following the vaccination a detectable protection was attained after

an interval of 14 days which repetition of the vaccination raised to a still higher degree. Experimentally, to be sure, premature lambing could not be prevented by the preceding vaccination, but in animals that were not pregnant the survival quota of vaccinated sheep attained 70 percent in contrast to a mortality of 70 percent in nonvaccinated sheep. The authors conclude from their findings that "the low degree of immunity produced by the antigen is sufficient to protect the sheep exposed to natural infections" and recommended vaccination in endangered herds.

Thus to date no one has been successful in developing a definite method for the prevention of listeriosis by active, passive or combined vaccination. But where the use of nonpathogenic strain, changes in the technique of inoculation, and longer duration of immunization procedures with stronger, more active concentrations might not perhaps lead to success, is still to be proved.

In prophylaxis one must immediately consider the old well known sanitary precautions. To this belongs, besides stable hygiene, also the protection of the animals against unusual influences of climate, and against chilling. In addition, food of high value should be given, although changes in the composition of the food of which among others Murray has always taken this into consideration, Graham and others believe this has no influence upon the course of

outbreaks of listeriosis. Sick animals should be isolated if possible. Since epizootics often vanish spontaneously, in order for it to survive until the next season it does not appear that these precautions are sufficient for the prevention of plague. Only the discovery of the natural reservoirs of the microorganism and the elimination of carriers of bacteria in infected herds will make possible a more workable prophylaxis which, however, appears to one today to be still far in the future.

6. Veterinary Police Procedures.

Listeriosis belongs to the diseases that are transmissible from domestic animals to man. To all appearances it is more prevalent in Germany than is commonly believed today (22s,294f,223,231,242).

Since the microorganism is pathogenic for people all corresponding legal provisions must find application with respect to listeriosis, that are in effect for example against Bang's disease or brucellosis, bovine tuberculosis, leptospirosis, and the various forms of blood poisoning as well.

It appears particularly necessary to broaden the legal provisions against the use of nonpasteurized milk for drinking (preferred milk) by consumers, so that this kind of herd must be free from listeriosis (and also from atypical listeriosis mastitis).

The possibility of an occupational infection for veterinarians, slaughterers, animal tenders, animal keepers, dairymen, and so forth

is apparent.

Special precautions should be taken in dissecting dead animals, above all in removing the brain and spinal cord. These tissues are highly infectious and comprise because of the proven pathogenicity for humans a real danger for the research worker. How great this is in practice, may not yet be stated, since the possibility of a Listeria infection has been studied little to date. In an American textbook on meat hygiene by Miller it was recommended that flesh of animals that had survived listeriosis, must, in any case, be inspected by the veterinary police and that the head of the animal must be rejected, even if there are otherwise no scruples against the use of the meat for human consumption to be made applicable. Definite instances of human infection by infected meat have however not been given publicity to date.

These recommendations do not take into consideration the fact that Listeria are still often found in other organs as heart, liver, spleen, intestines, perhaps also in the masseter muscles, particularly in illnesses without involvement of the CNS.

If also in artificially infected animals Listeria cannot be detected in the affected organs after more than 5 days following slaughter, Listeria-infected animals should not be used for meat, nor should their meat be used commercially, even though no detectable macroscopic changes are found at meat inspection (871).

The brain and medulla of spontaneously recovered animals who had encephalitis are on the contrary to be considered free of living bacteria 3 weeks after the healing.

The transmissibility (or possibility of transmission) thus found necessitates consideration, in Germany, of an extension of the laws on meat inspection. As a macroscopic detection of the disease is not feasible, bacteriologic study of the meat must be carried out in case listeriosis is suspected. Schimke gave this question complex a distinct place; he demanded that listeriosis be excluded, with full justification, as a source of fatal disease. In case of proved disease the whole animal carcass should be rejected immediately according to Section 32, paragraphs 1, 7 of the "AB.A." of the meat inspection law as unfit for human consumption (and also unfit for other animals). Listeriosis no doubt falls under the classification of the bacterial blood poisonings, so that the existing provisions for this can also now be brought into use. According to certain publications (see above) the meat should be considered fit for limited use according to section 36 paragraph II item 3.

The duty of reporting suspected or real cases of listeriosis can hardly be circumvented in view of its significance in human medicine. The cooperation of the organizations of the public health

service with the veterinary police research boards and animal health offices is important so that practising physicians may be informed immediately in case the disease appears. This is all the more necessary, since most doctors know little or nothing about the various forms of the course of listeriosis in humans.

Conclusions.

L. monocytogenes is the source of sporadic, enzootic and epizootic diseases in numerous small and large animals. It occurs now as a sepsis, again as an isolated disease of an organ with various degrees of CNS involvement.

Septic disease appears predominantly in rodents, fowl, and young animals. Central nervous system involvement is found more often in adult large animals. This rule is not without exception. Atypical and clinically quiescent forms are not seldom found. The degree of maturity of the organism and the latent infection apparently play, in addition to the port of entry, a decisive role in determining the course of the disease.

In the case of infected pregnant animals there is, with increasing frequency, fetal damage, fetal death, and abortion. Listeriosis as a cause of premature calving and premature lambing needs further study. The mortality from clinically manifest disease is high. Disease outbreaks in valuable breeding animals and herds of cattle may lead to severe

economic hardship because of them. Listeriosis is found in animals throughout the world, and will be recognized not only in Germany, but also in many other lands, with increasing frequency. The number of cases diagnosed to date runs into the thousands.

Since a clinical diagnosis is not definitely successful, and manifold possibilities for confusion exist, the outlook for treatment is poor. Sulfonamides and tetracycline promise the best therapeutic results in the case of immediate and maximal dosage with them. Prophylactic immunizations have to date shown no definite protective activity.

The epizootiology is still largely unknown. Contact infection, aerogenic transmission, infection while covering, rhino-and oto-genic pathways for development, have been considered, without man learning exactly which way yet. The natural reservoir of microorganisms is supposed to exist among rodent and fowls.

Regarding its definite pathogenicity for humans (see Section C) a danger exists for all persons who have to do with animals or animal products, especially, meat, game, unpasteurized milk and milk products. The food laws must, therefore, in the future also take listeriosis into consideration.

C. Human Listeriosis.

I. Incidence and distribution.

The significance of infectious diseases that are chiefly known as zoonoses from their behavior, is becoming increasingly important in human medicine. It should be remembered that only during the last three decades has the role of salmonelloses, pasteurelloses, leptospiroses, brucelloses and lately toxoplasmosis in the genesis of human disease been correctly understood. Corresponding to the current improvement in understanding of etiology the number of clinically inexplicable, infectious processes remaining diminishes; and, too, serious postinfectious complications may now be explained frequently by the causal agent (28, 91, 100, 135, 176, 193, 213).

This does not ultimately apply to listeriosis in humans, one of the latest infectious diseases caused by bacteria to become known. The nomenclature related to it was discussed on page 37 (of the text). While up to 1945 hardly 20 cases were reported, the number of definite findings has increased nearly tenfold during the short period of time since then. But these probably include only a small fraction of the true number of patients afflicted.

Listeriosis as a human disease was first diagnosed in Germany, for example, early in the year 1951 at the Health Institute of Bonn. Soon the immediately popular view that it is a question of a very rare

disease must be revised (there were up to that time about 100 proved cases in the world literature). The first reports (133, 242a, g,h, 106,210,157c) were followed in all parts of Germany by so many findings of human listeriosis that already in the spring of 1955 about 100 further cases had been bacteriologically confirmed. If one adds to the patients included here the ones that were probably infected, on the basis of serologic findings, the number then rises to a few hundred.

This fact is in itself surprising in a land that has so many well-equipped research institutions, and may only be explained in that the Listeria were practically unknown to the persons devoted to the practice of human bacteriology. By reports in pathologico-anatomic publications it is proved that a few important course-forms of human listeriosis are not rarities; nevertheless, without being any more successful than formerly in gaining insight into the nature of the disease.

The increase by leaps and bounds, during the course of the last three years, is thus not equivalent to a true increase in listeriosis morbidity. This was perhaps--particularly among the farm population--hardly any lower before that. We believe that the considerable rise in the number of reports not only results from better understanding of the microorganism, but also should be viewed as a result of the general increase in bacteriologic investigations, the yield of which

may be considerably increased by the development of improved methods of culture and of differentiation.

That it is really not a question of a new uprising of an infectious disease is demonstrable also in that reports of disease syndromes similar to listeriosis or similar bacteriologic findings that are compatible with Listeria-infections, however else they have been explained, have been revealed in an astonishing fashion.

This is especially true for the so-called "pseudotuberculosis" (109,199,216,238,242a,267 and others) and meningeal inflammations from Acidobacteria, diphtheria microorganisms and other Corynebacteria, but also for a series of febrile, disease syndromes with concomitant glandular swellings, of unclear etiology. Even where one holds their connection with listeriosis still in reserve, and believes further research is necessary, for example in the evaluation of the much discussed miliary necrosis of organs in infants and in newborns, the role of Listeria-infections will no longer be contested.

It is as good as certain that at least the overwhelming majority of the reports set forth in Table 9 in reality resulted from nothing other than human listeriosis under other names.

Listeria are basically different from the microorganisms of many other zoonoses in that their transmission to people is connected with no changes in the symptomatology of the disease, similar to the behavior of Pasteurella and Leptospira. Therefore it causes the same clinical form in men as in the listeriosis observed in animals.

Table 9. Human infections in which the microorganism may perhaps have belonged to the Listeria group.

Year of the disease	Number of cases	Clinical form	Age-group	Outcome	Nation	Bacteria detected	Authors
1891	1	Gastro-enteritis	adult	fatal	France	Gram-positive bacilli	Hayem
1893	1	Pseudotubrc.	newborn[1)]	fatal	Germany		Henle
	1	Pseudotberc. & Meningitis	newborn[1)]	fatal	Germany	Gram-positive bacilli	Henle
1901	1	Pseudotubrc.	premature	fatal	Germany	Gram-positive bacilli	Aschoff-Wrede
1912	1	Pseudotbc.	newborn	fatal	Germany		Aasler
1913	2	Malignant granuloma	child	fatal	Holland	Corynebact.	de Negri
1915	2	Pseudotbc.	infants	fatal	Germany		Schneider
	5	Meningitis	infants	4 fatal 1 recovered	Australia	Diphtheroid	Atkinson
1919	1	Meningitis	adult	fatal	USA	Diphtheroid	Dick
1921	1	Pseudotbc.	infants	fatal	Germany	Gram-pos. bacilli	Fraenkel
1923	2	Pseudotbc.	newborn	fatal	Germany	Gram-pos. bacilli	Fraenkel
	1	Pseudotbc.	child	fatal	Germany	Argentophil bacilli	Kantschewa
	1	Pseudotbc.	infant	fatal	Austria		Konschegg
1924/25	3	Pseudotbc.	infant	fatal	Germany	Gram-pos. bacilli	L. Schwarz
1925	5	Mononucleosis	adults	recovered	USA	Diphtheroid	Baldridge
1927	1	Pseudotbc.	infant	fatal	Switzerland	Argentophil bacilli	Werthemann
	2	Miliary necrosis	premature twins	fatal			Schleussing
1929	1	Meningitis	adult	recovered	USA	Diphtheroid	Kessel
1931	2	Pseudotbc.	stillborn newborn	fatal	Switzerland	in one case, Gram-pos. bacilli	Iff
?	1	Pseudotbc.	infant	fatal	Germany	--	Kaufmann
1937	1	Pseudotbc.	newborn	fatal	Germany	--	Oestern
1937-53	11	Miliary liver necroses	newborns and infants	fatal	Germany	Argentophil bacilli	Frobosse and Schmitz

(continuted on next page)

(Table 9 continued)

1939	2	Meningitis	adult	fatal	Austria	Acidobacteria	Kuchinka
1940	1	Meningitis	child	fatal	Germany	Diphtheria-like bacteria	Kalbfleisch and Kretschmer[2]
1946	1	Septic granulomatosis	newborn	fatal	Germany	Acidobacteria	Lodenkämper
1946	1	Pseudotbc.	infant	fatal	Germany	--	Oebike
1948	2	Fetal sepsis	newborns	fatal	Germany	Gram-positive bacilli	Staemmler
1949	1	Pseudotbc.	infant	fatal	Germany	--	Stockmann
1951	1	Pseudotbc.	premature	fatal	Germany	--	F.T. Brandis
1949/50	4	Hepatitis & sometimes cirrhosis	newborns and infants	fatal	England	--	Dible, et al

[1] Twins, both of which were diseased and dead.

[2] At that place, further afflicted cases discovered by Reiche, Glaser, et al.

Table 10. Bacteriologically proved cases of listeriosis in humans

Year of disease	Number of cases	Clinical form	Age-group	Outcome	Nation	Authors
1918	1	Meningitis	adult	fatal	France	Dumont and Cotoni
1929/30	3	Mononucleosis	adult	recovered	Denmark	Nyfeldt
1932	1	Conjunctivitis	adult	recovered	Austria	Anton
1932	1	Meningitis	child	fatal	Holland	Kapsenberg
1933	1	Septic granulomatosis	newborn	fatal	USA	Burn
	1	Meningo-encephalitis	adult	recovered	USA	Schultz, et al. Marcellus, et al.
1934	2	Meningitis	infants	1 fatal 1 recovered	Holland	Kapsenberg
	3	Septic granulomatosis sometimes with meningitis also	2 newborns 1 adult	fatal	USA	Burn
	1	Meningitis	adult	fatal	USA	Allen
	1	Meningitis		fatal	Norway	Tesdal
1935	1	Meningitis	child	recovered	USA	Carey
	1	Meningitis	adult	fatal	England	Gibson
1937	4	Mononucleosis sometimes with meningitis	adults	recovered	Denmark	Schmidt & Nyfeldt
	1	Meningitis	child	fatal	USA	Poston, et al.
	1	Meningitis	adult	recovered	Uruguay	Porzecanski and de Baygorria
1938	3	Glandular fever with mononucleosis	adults	recovered	Denmark	Nyfeldt
	1	Meningitis	child	recovered	USA	Wagner and Porter (cited 201)
1939	1	Meningitis, otitis media	infant	fatal	England	Wright & MacGregor
	1	Meningitis	adult	recovered	Holland	Kapsenberg
	1	Angina, mononucleosis	child	recovered	USA	Pons & Julianelle
1940	1	Meningo-encephalitis	adult	recovered	Argentina	Savino
1941	1	Mononucleosis	adult	recovered	England	Webb
1942/45	11?	Meningo-encephalitis		9 fatal 2 recovered	USSR	Gudkowa & Sacharoff

(Table 10 continued)

1943	1	Meningitis	adult	recovered	France	Harvier, et al.
1944	2	Granulomatous conjunctivitis	adult	recovered	USA	Felsenfeld
1946	1	Meningitis	child	recovered	USA	Handelman, et al.
1947	1	Meningitis	adult	fatal	France	Martin, et al.
	2	Meningitis	child adult	fatal recovered	France	Sedallian, et al.
	1	Typhus-like picture	adult	recovered	France	Sedallian, et al.
	1	Meningitis, pneumonia	adult	fatal	Australia	Stanley
	1	Sepsis, meningitis	infant	recovered	Holland	Van Driest
	1	Meningitis	infant	fatal	Holland	Beute, et al.
	1	Meningitis	infant	fatal	Holland	Slooff
1948	1	Meningitis and conjunctivitis	adult	recovered	Holland	Beute, et al.
	1	Meningitis	?	?	USA	Wheeler
	1	Fever after caesarean section, glands swollen	adult	recovered	Cuba	Felsenfeld
1948/49	18?	Angina, with sepsis sometimes	adults	fatal some recovered	USSR	Schamesow
1949	12	Angina, generalized infection and conjunctivitis	adults	recovered	USSR	Bilibin
1949/54	2	Meningitis	1 adult 1 child	recovered	USA	Jaeger & Myers (319)
	1	Sepsis	newborn		USA	"
	1	Clinically OK	adult	recovered	USA	"
1950	1	Meningitis	adult	recovered	USA	Berry
	2	Meningitis	newborn	1 fatal 1 recovered	USA	Line & Cherry
	9[1)]	Conjunctivitis & swollen glands	8 children	1 recovered	USSR	Pletnewa & Stiksowa
1950-54	48	Granulomatosis infantiseptica, meningitis, and pyelitis	newborns & adults	fatal 3 recovered	Germany	Reiss, et al., Erdmann & Potel,
1951	1	Granulomatosis septica, poly-serositis	adult	fatal	Germany	Seeliger, et al.

1) see page 70

(Table 10 continued)

1951	1	Meningitis	premature	recovered	USA	Line & Appleton
	1	Meningitis	child	recovered	USA	Bennett, et al.
	1	Meningitis and mononucleosis	adult	recovered	Germany	Seeliger and Leineweber
	1	Meningitis	adult	fatal	Germany	Hein
	1	Meningitis, pneumonia	adult	fatal	Norway	Ödegaard, et al.
	1	Meningitis, pyelitis	infants	fatal	USA	Bergstrom
1951/52	3	Meningitis, encephalitis	adults	fatal	USA	Gray (308) Ferguson (304); Williams and Hornung
1952	1	Pneumonia, sepsis	newborn	fatal	Germany	Linzenmeier, et al.
	?	Oculoglandular form			USSR	Shmelova
	1	Meningitis	adult	fatal	USA	Winkler & Carter
1950/52	3	1?	?	?	Canada	Allin,
		1 conjunctivitis	newborn	?		MacDonald, cited in
		1 granulomatosis septica	newborn	fatal		Roy (317)
	5	Urethritis	adults	recovered	Argentina	Wenkebach
1952	2	Meningitis, pneumonitis	adults	recovered	USA	Finegold, et al.
1953	1	Meningitis	?	recovered	USA	Binder, et al.
	1	Meningitis sepsis	adult	fatal	USA	Schulze, et al.
	13	Granulomatosis septica	infants	10 fatal 3 recovered	Germany	Martinek (316,344)
	6	Meningitis	adults	5 recovered 1 fatal	Germany	Boese (301,346)
	2	Meningitis	child	1 fatal 1 recovered	Germany	Rische (325).
	2	Encephalitis, meningitis	adults	fatal	Germany	Fischer (305)
	1	Granulomatosis septica	infant	fatal	Germany	"
	about 26	Pfeiffer's disease		recovered	Germany	Schabinski (326)
	2	Suspected as diphtheria	adults	recovered	Germany	"
	1	Meningo-encephalitis	adult	fatal	Germany	"

(Table 10 continued)

1953	2	Cryptogenous sepsis	adult	1 fatal 1 recovered	Germany	Schabinski (326)
	1	Sepsis	adult	fatal	Germany	Brandenburg (302)
	2	Inexplicable fever[1]	adults	1 recovered 1 fatal	Germany	Hoffmann (309,333)
	4	Meningitis, granulomatosis sept.	1 adult 3 newborn	3 newborn fatal, 1 recov.	Germany	Kröger (310,338)
	1	Meningitis	child	fatal	Germany	Preuss (324)
	1	Meningitis, encephalitis	adult	fatal	Germany	Böhmig (300)
1951-53	25	Granulomatosis septica	newborns	fatal	Czecho-slovakia	Patočka (322)
1952/53	12	Sepsis, pneumonia, meningitis	prematures stillborns	fatal	Germany	Hahnefeld & Nisolk; Hagemann & Simon
1953/54	1	Encephalitis	child	?	Austria	Flamm et al.
	1	Meningitis	child	recovered		
	1	Meningitis	adult	?		
1954	1	Meningitis	infant	recovered	England	Baar & Rogers (299)
1954	1	Meningitis	infant	fatal	Germany	Kiehl
	1	Meningitis	newborn		Germany	Lodenkämper (334)
	1	Disease of the CNS	adult		Germany	Sonnenschein (330)
	1	Meningitis	adult		Germany	Schefczik (327)
1952-54	3	Meningitis	1 adult 2 newborns	fatal 1 recovered (newborn)	France	cited in (332); Andrieu
1952/53	3*	Psychoses	adults	recovered	USSR	Timofeeva et al.

1) and endocarditis

* only detected serologically.

Appendix to Table 10:

Meanwhile the number of proved cases of listeriosis has steadily risen higher; in Leipzig alone at least six cases were proved bacteriologically and eleven further cases probably determined on the basis of the classic anamnesis in connection with the serologic findings (323,337). Within the borders of the (West?) German Republic during 1953/54 at least 20 cases with

previous histories that were typical and definite serologic findings, that were compatible with a diagnosis of listeriosis (242j) and a somewhat smaller number in the DDR (East Germany?) (323). Further listeriosis cases were revealed by findings in the case of a newborn with icterus in Montreal (Prissik), in adults with meningitis in Toronto (Greey) and Vancouver (Cockcroft), lately too in New Haven, (Haley) (cited about 317)). In Hamburg also two cases were observed (158a) the same as in Washington (347), in newborns in Czechoslovakia (350) and further in Moers, Lower Rhine, was found a woman with post-partum tuberculous meningitis and listeriosis meningitis (Boese and Worth (301)), in Vienna in a child (349), in Bonn in many small children with cerebral involvement (Lang) and in London in a 64 year old man with encephalitis (Kopplow and Endred (315a)), lately in Munich too in a syphilis patient who died of meningitis (Poetschke) as well as in a case of meningitis in Marburg (Nieth and May).

The global distribution demonstrated in the animal kingdom finds a parallel in its existence among man, whereby repeated individual cases of human listeriosis first called attention to the disease among animal herds that obviously was heretofore unknown.

Since because of the swift increase in the volume of literature on listeriosis, in recent times, a review will soon be no longer feasible, it appears pertinent to bring together the bacteriologically proved human cases that have been published up to the end of 1954 (Table 10).

It is still too early to make pronouncements on the frequency, since in humans we today still know too little about the morbidity.

When the cases appear it is usually, with few exceptions, a question of a sporadic incidence. To be sure, a few groups of patients

may thereby be pointed to (10,40) in regional increases (95,191,204) as well as true epidemics (227,326), which show that the epidemic appearance of the disease is quite possible, a fact that Webb was among the first to point out.

The most pertinent findings result with persons who have direct or indirect contact with animals. To these belong, lastly, also those who use animal products, for example, uncooked milk. All appearances have been among farm folks and also veterinarians, animal keepers, breeders, among whom the environment is more favorable for the development of listeriosis than, for example, among city folks. However it is too early to be able to pass judgement since we know still very little about the epidemiology and pathogenesis of listeriosis. Above all, it is not known whether or not human beings themselves can come under consideration as reservoirs of the micro-organisms.

There is no particular susceptibility of either sex, obviously, if we disregard the peculiarities of listeriosis in pregnancy (see p. 86 of the text).

Also, one cannot speak of a strong correlation with the age of the victim, since the cases published to date fall among all the age-groups. To be sure, inasmuch as limits must be drawn, one can distinguish among the clinical forms certain apparent differences

as to the ages in which they will appear.

While in fetuses, newborns and infants a septic-granulomatous syndrome predominates, the disease often manifests itself, among children and adults, by marked central nervous system involvement. This rule is by no means without exceptions, since also in adults the same organic involvements have been observed as in the earlier years of life.

However, it is obvious that the body of the adult is no longer so defenselessly exposed to the infection by listeriosis bacteria as during the first months and years of its existence. This also finds a parallel in the animal kingdom. Possibly this capacity of resistance depends upon a latent infection, that likelihood being obviously stronger than we currently surmise. Perhaps it is therelative immaturity of the young, particularly however, the newborn organism or that existing shortly after birth, which appears to be predestined in some way to numerous bacterial infectious diseases, to which also listeriosis belongs. On the otherhand, the percentage of fatal listeriosis cases among older people (see table 10) is higher than among the middle aged, which leads us to suppose that changes in the capacity for resistance as are observed in the extremely old, results in the appearance of a severe course for listeriosis infections.

2. Clinical aspects of listeriosis in man.

The clinical symptomatology of human listeriosis is decidedly many-faced, so that the clinician in the case of patients with the most divergent symptomatology must think about the possibility of listeriosis.

According to its course we may differentiate:

a) acute to hyperacute

b) subacute

c) chronic-protracted and

d) abortive

forms of the disease.

The onset is always unknown and the prognosis in untreated cases often from dubious to hopeless if it is a question of an acute or subacute disease of the C.N.S. Usually however, the forms of disease that were acute in the beginning go on to be cured or carry over into a chronic stage. Abortive forms of the disease have been to date mostly recognized in pregnant women, where it is demonstrated that the same microorganism that barely appears able, in the mother's body, to cause any clinical symptoms, can cause in fetuses or in newborns or infants a hyperacute, almost always fatal infection. Many times a subacute course predominates from the onset of clinical symptoms, that is possibly conditioned by a specific defense mechanism or a condition of immunity of the patient.

The clinical phenomena show often, but yet not always, a certain correlation with the above described forms of the disease. It appears expedient that the clinic should regard listeriosis as one of the most important disease syndromes.

a) Septic sore throat form with mononucleosis. Notwithstanding a few earlier reports (see tables 9 and 10) Nyfeldt has had the honor of being known as the man who discovered the Listeria as a source of human disease.

In 1929/30 he was able to isolate these bacteria from the blood of three patients that were clinically ill with the symptoms of listeriosis. On the basis of careful hematologic and experimental studies he named L. monocytogenes as the causative organism of listeriosis. (Pfeiffer's glandular fever, monocytic angina) and published his theory in eight other reports on listeriosis due to Listeria, including the small milk-epidemic in Vedbaek (227,320).

All patients had severe sore throat, sometimes with the picture of a peritonsillar abscess, with gray-white coated tongues, glandular swellings, fever of about 38-39°C. and generally a leukocytosis with mononucleosis (up to 70%). A few cases were complicated by meningeal symptoms. The etiology was confirmed by isolation of Listeria from blood and spinal fluid. The course was favorable. -- The association of listeriosis with meningoencephalitis and meningitis had been already

reported previously by Hecht-Johansen, Epstein, Dahmshek, Sucher and Schwarz (227).

These publications were hardly observed immediately when there arose widespread contradictions, sometimes even amounting to flat challenges, since it happened that many researchers were not successful despite many attempts to affirm that the findings were due to other diseases.

The scientific opponents of the theory of Nyfeldt call attention thereby among other things to the inability of the Listeria, in animal investigations, to activate the formation of heterophil antibodies.

By the work of Paul and Bunnell it was shown that agglutinins against red blood cells of sheep frequently--but certainly not always--appear in the serum of patients with listeriosis, of which the detection at serum dilutions of 1:64 and over (sometimes a titer of 1:160 is given as the limiting value) has attained great significance in the diagnosis of listeriosis. These heterophil antibodies are different from the ones that appear after injection of animal serum ((Hanganutziu, 1924; Deicher 1926). For summaries see Lippelt and Nogalski (1953) as well (218)). They were observed in about a third of the cases.

Stanley was able to show, that neither the protein nor the

polysaccharide nor lipoid fractions of L. monocytogenes are able to agglutinate sheep erythrocytes or to stimulate the formation of heterophil antibodies in rabbits. Heterophil antibodies could likewise not be precipitated by absorption of the serums with Listeria antigens.

Wising (1942) found in 27 patients just as few Listeria in blood or excised lymph nodes as Becker (1931) in the acute stages; in serum from convalescents no Listeria agglutinins were detectable. Also the injection of killed suspensions of Listeria into volunteer human research subjects did not lead to the formation of heterophil agglutinin. Similar findings were made by Janeway and Dammin (see also (282)); Sohier (250c) as well as Harvier and coworkers likewise rejected the connection between Listeria-infections and listeriosis.

This is in contrast to further bacteriologic observations of unequivocal proof of the presence of Listeria infections in case of glandular fever and monocnucleosis (198,281a,326).

Perhaps the case of Baldridge and others (1926) belong to this group also.

A look at the case history of one of the cases diagnosed by Webb might serve to clear this up:

It concerns the case of a 20 year old medical student with a febrile infection that began with glandular swellings in the neck

which were not particularly painful. Mild chills, muscle pains, frontal headache and general feeling of being ill vanished in a few hours. After a few days of bed rest the fever subsided. When the patient got up on the eleventh day of illness, a prompt relapse resulted. The initial symptoms were again aggravated, and together with painful cervical glandular swellings appeared a lymphadenitis of the inguinal nodes. There also was an acute pharyngitis. The blood count showed on the 14th day of illness typical changes after the manner of anlisteriosis with 87% lympho- and mono-cytes in a cell count of 17,800. All symptoms receded steadily during 4 weeks bed-rest, and in the fifth week the glandular swellings had also disappeared. General weakness and tiredness persisted, however, for 2-3 months more.

On the 14th day of sickness success was obtained in culturing L. monocytogenes from venous blood.

The Paul Bunnell test was positive at the same time at a serum dilution of 1:2048. Previous injections of horse serum had not been performed. On the contrary, formol-antigen of the Listeria strain were agglutinated, on the 37th day of illness and 12 months later, at a serum dilution of 1:25.

Further reports on an anginal-septic course of listeriosis with increase in monocytes were made by Bilibin. He observed in Russia

12 instances of an acute infectious disease in persons who lived in an environment heavily infested with mice.

The clinically decisive syndrome consisted in the acute beginning of chills, headache and muscle aches, fever, facial hyperemia, conjunctivitis, and a widespread rash with roseolas, particularly about the face and extremities. Thereupon came enlargements of the lymph nodes, particularly in the cervical region; more or less extensive liver and spleen edema, pharyngitis, purulent or pseudomembranous angina follicularis (sore throat) and stomatitis, concomitently also diarrhea. The rate of blood cell precipitation was slightly accelerated or was normal. The blood picture showed only a mild leukocytosis and a more or less definite monocytosis.

The Paul-Bunnell test was negative in all cases, the Wassermann reaction in one patient was transitorily strongly positive; in three cases the culture of L. monocytogenes from the organs of guinea pigs that had been inoculated with the patients blood was successful, while direct cultural investigations were without result. Listeria antibodies were detectable in the blood of all patients during the third week, attaining titers of 1:500 and over, and remaining unaltered for many months.

Concerning the general involvement of the tonsils with

pseudomembranous angina follicularis, glandular swelling, and monocytosis reported by Urbach and Schabinski in an institutional epidemic among student nuns in which about 26 became ill (349a). Clinically it was a question of a typical epidemic of listeriosis. From throat smears and water that these patients had gargled, in many of these cases, L.monocytogenes was isolated.-- In contrast to this remains the series of investigations on 3500 throat smears for Listeria in patients with listeriosis, tonsillitis, diphtheria, etc., in Australia, which yielded no results (253d).

Regarding the possibility of a serologic delination of the Listeria-monocytic-angina follicularis from other listerioses the opinions are divided. Meanwhile it is certain that in many patients with listeriosis the Listeria titer rises during the course of the disease, and persists at many degrees of dilution (31,152a,b, 179d,e, 227,281a). In investigations on 20 patients three groups were formed on the basis of results of the Paul-Bunnell test and the Listeria agglutination reaction (253a):

Table 11: Subdivision of listeriosis following serologic investigation (according to Stanley)

Group	Paul-Bunnell test	Listeria agglutination	Percentage
A	positive	positive	35
B	positive	negative	35
C	negative	positive	30

Stanley inferred that the diseases in Group A probably resulted from an infection with L. monocytogenes.

Of course, such a hypothesis is based on the assumption that the Listeria titers are specific in humans, that is, they are definite indications of a previous immunologic exposure. However, final proof of this is still lacking (see page 119 ff).

In investigations on sera of healthy persons and listeriosis patients by means of the Widal test and the complement fixation reaction, were found, in some instances, Listeria antibodies and deviations of complement at low serum dilutions (188b). The Paul-Bunnell test was positive in these cases.-- On the contrary control studies carried out elsewhere showed no significant differences among a sizeable amount of material (242c).

In the institutional epidemic at Jena were obtained high titers of up to 1:1600 against O- and H-antigens of the strain of the micro-organism belonging to type 4, and indeed not only in the clinically

severely ill students but also in the remainder of whom a few had survived a kind of grippe with angina follicularis (326). The titer was observed to follow a curving course and later became considerably diminished (349a).

The serologic findings thus varied considerably from one another, but must not, however, be unreservedly discounted. The studies were conducted at different places and at different stages of the disease. The research technique itself is in no way standardized. Since practically every researcher worked with antigens prepared by others, the results are generally not comparable.

Stanley's conclusions are based, for example, on rises in titer in relatively low serum dilution (1:4-1:128); other authors began their series of dilutions at 1:20. The antigenic differences among the various Listeria types were often not taken into consideration.

Hematologically listeriosis is characterized by typical changes in the white blood cell picture, that under natural conditions and also in animal experimentation may also be caused by Listeria (see p. 25). Therefore it is hardly to be expected that hematologic investigation would lead to valuable results in answering the question of Listeria-infection--virus genesis.

It may be considered as proved that the etiology of listeriosis is not unique, but under this clinical syndrome hides infectious

diseases of different origin (85,105,114,233,342). If the meaning is also taken that clinically and serologically a differentiation between listeriosis, monocytic angina follicularis, and other lymphadenoses is possible (177) then the concepts of the clinicians come to be regarded as generally synonymous (85).

Generally the trend is becoming widespread--as determined by the results of far reaching experimental and clinical research--to consider one or more kinds of viruses as essential microorganisms causing listeriosis. Individually, these works are to be found in the reviews of Murray and Girard, Nogalski and Lippelt as well as Jorke (Zeitschr. f. ges. Inn. Med., 1953, p. 687).

This however does not exclude the possibility that Listeria may be able to cause clinically the same or at least confusingly similar phenomena. This anginal--septic form of listeriosis is generally found in adults and only occasionally in children. If definite increases in monocytes appear, the disease syndrome may hardly be distinguished from listeriosis caused by viruses. Not always are all clinical symptoms present. As already explained, there are cases with only a limited monocytosis (31). From further observations (68,309,333) it is to be concluded that there are abortive course forms with and without monocytosis as well as with and without angina follicularis which give the clinical impression of an inexplicable

febrile infection, in which the detection of Listeria in the blood is successful. Transformations into the meningitis form are likewise known, beginning with mild signs of meningeal irritation with Listeria-negative or -positive spinal fluid, and up to severe, purulent meningitis in connection with a syndrome of grippe with glandular swellings and monocytosis (242h).

Since it is today settled that the Listeria were the organisms of a causative nature in a number of patients with a picture of listeriosis, further cases may be hidden among Paul-Bunnell positive as well as negative disease stages.

Bacteriologic investigations are indispensable for clarification of this. These must be carried out as early in the acute stages of the disease as possible, and by means of repeated blood cultures from culture specimens from the swollen lymph nodes. If also throat smears were often negative, (253d) the observations in the epidemic in Jena yet show that the detection of the causative organism is possible by this method (326). In many cases obviously the artificial medium was at fault, in case of the detection of only a few bacteria, so that we are dependent upon the biologic investigation (injection of the patient's blood into suitable research animals) (31).

Serologic investigations allow only a posteriori conclusions to be made, on the etiology of listeriosis if for example the Paul-Bunnell

test or the Widal Listeria reaction result positively alone in diagnostic dilutions. Results such as those of Webb or in Stanley's group A lead to the suspecting of a double infection.

The absence of Listeria-titers in repeated control tests speaks against an infection with these bacteria, against which antigens of human and animal bodies relatively easily form agglutinins. Moreover, positive results must be critically evaluated with respect to the frequent finding of Listeria-titers in control subjects (see page 119 ff).

The widespread involvement of the area about the throat with affection of the cervical lymph nodes points out the probable point of entry. The other phenomena may be attributed to a lymphogenous or hematogenous spread.

The prognosis of the listeriosis or monocytic angina follicularis caused by Listeria is favorable. The course was generally very promising in the cases reported, with the exception of a 35 year old man who died of a septic Listeria pneumonia with abscesses on the tonsils, the nasal septum and the oral mucosa (222).

Just as in the forms of mononucleosis due to viruses, with conservative treatment in three to four weeks generally only spontaneous healing occurs, with a convalescent period of many months duration being needed.

b) Oculo-glandular forms. During the course of an anginaseptic listeriosis a conjunctivitis also often appears (31). This is viewed as an indication of a generalized infection.

To be described here is a localized, acute to subacute inflammation of the conjunctiva as we also see in experimental conjunctival infections of research animals (see p. 32). Luck is to be properly thanked for the Anton test, in that it originated when he inadvertently squirted a technician in the face with living Listeria, and a specific purulent conjunctivitis resulted, that healed without complications after treatment with Protargol.

This kind of Listeria-conjunctivitis traceable to natural infection has been reported many times in the USA and the USSR (68b,196,246).

In a Chicago fowl market in 1944 two employees who had been cleaning the fowls became ill, with a follicular, hypertrophic conjunctivitis which did not respond to the usual treatments. In one patient the inflammation had already affected the cornea, of the left eye, where it caused a smooth ulcer with irregular borders.

Biopsy specimens showed histologically the picture of the Listeria-conjunctivitis in animals, so that it was concluded that it was a Listeria infection. The suspicions were confirmed by the frequent finding of the microorganism in the spleens of slain hens in the same market.

Transmission was obviously by way of an infection from filth.

In eight children and one adult, who had contact with animals ill with listeriosis, Russian authors reported (196) an oculoglandular form of the disease, which manifested itself in the form of a conjunctivitis with widespread swelling of the parotid and submandibular glands therewith occurred sometimes a fever of up to 40°C. and a relative lymphocytosis up to 70%. The incubation time, that we even in human listeriosis do not yet exactly know, is reported as from 3 to 45 days, the duration of the disease 30 to 100 days. Specific agglutinins are formed regularly and may be detected with a diagnostic test for Listeria in serum dilutions of 1:100 to 1:5000 even after a year. The diagnosis is made on the basis of seroanalytic findings. It allows one to exclude definitely Bang's disease and tularemia.

Localized Listeria-conjunctivitis does not always heal spontaneously; but can also be succeeded by a purulent meningitis (lymphgenous?) which ends fatally (26).

c) Septic--typhoid-like course forms. If sore throats are not present, and instead a highly febrile general infection predominates, we may speak of a septic-typhoid-like form. Only a few cases of this kind have been described to date, if one disregards the generalized infections under the granulomatosis cases, which will be taken under special consideration later on.

A pertinent case in an adult was reported by Sedallian and his coworkers. During the course of the disease there developed, apparently from a spread from foci in the lungs, a widespread pleural empyema. Healing occurred only after a long hospital stay following a pleurotomy.

In many respects it resembles the case of a Norwegian farmer who infected himself while cleaning his sheepfold and died from a septic meningitis (181).

On section a generalized, focus-forming, purulent broncho-pneumonia was found in the left lung. Listeria were cultured from the foci. The infection may have resulted aerogenously from bacteria-laden dust since listeria were discovered in numerous investigations in the sheep fold of the dead man.

Thereafter similar cases must be suspected as in the epidemiology of Q fever, in which Coxiella burneti by their chief host, the sheep, are transmitted aerogenously to man (265).

The fatal case already mentioned in an adult (222) fits into the picture of this section.

A clear division into septic-typhoid-like and anginal (sore throat) septic course form is not always possible, since the symptoms may overlap.

The appearance of unknown foci (sinusitis, otitis, etc) results also in, for example, a picture of a cryptogenous sepsis in listeriosis,

in the course of which detection of the microorganism from the blood or urine is successful (326).

It is to be remembered that this kind or similar cases will be still more frequently found, as soon as more attention is given to the presence of a possible listeriosis and Gram-positive bacilli in blood cultures are not looked on as secondary impurities, as so often happens (compare 74).

Listeriosis of the CNS: As a result of a generalized infection or spread of local processes in the nose, throat, eyes and middle ear, there occurs not seldom an instigation or commencement or sowing of Listeria into the meninges, brain and spinal cord. This leads to a purulent meningitis or encephalitis frequently also to a meningo-encephalitis or formation of a brain abscess.

No age group is immune against this most dangerous form of listeriosis. The published literature contains case histories of this from early childhood to the most advanced ages of life, of course it is to be remembered here that meningoencephalitic diseases in very young and very old people are frequently reported as other course forms.

The involvement of the central nervous system was brought into a relationship with a neurotropism of the microorganism. Whether we will perhaps find a true neurotropism in the case of Listeria is

uncertain. In case of Listeria the infection of the central nervous system is in many instances a partial result of the septic generalized infection, similar to Coli and tubercle bacilli infection of the meninges in childhood then at autopsy the microorganisms are found not only in the spinal cord and brain, but also in the liver, spleen, kidneys, blood and heart muscles. There are however cases in which the CNS is isolatedly afflicted.

Meningitis due to Listeria cannot be differentiated clinically from meningeal inflammations due to other sources and can be diagnosed definitely only by the use of bacteriologic methods.

Individual observations indicate that in cases in which the search for the microorganism was not successful or was at the time of investigation no longer successful, serologic methods (Widal test and complement fixation test) may give valuable hints in critical differential diagnosis. High titers as well as typical titer curves will repeatedly be observed in bacteriologically confirmed cases. Spinal fluid agglutination is detectable only at the lowest serum dilutions (up to 1:10) (242j).

In newborns and infants the symptoms at onset are not characteristic.

Immediately there are as the only detectable indications of an acute disease with shallow and limited breathing, slight cyanosis, fever, and refusal to eat. The appearance of convulsions, muscle twitches,

sudden cessation of breathing, with severe cyanosis, increased irritability, and increased fontanelle tension indicate the infection of the CNS, which with increasingly severe cerebrospinal meningitis, lethargy, or delirious stages, is generally fatal.

In small children the onset of an encephalitic irritation phenomenon is commonly indicated during the course of listeriosis by regularly occurring fits of rage (151).

The disease begins in adults with a more or less widespread often grippe-like prodromal stage that is stormy, with severe headaches, chills, fever, increasing neck rigidity, retching and vomiting, and leads to death after periods of increasing somnolence broken by episodes of agitation. The course is often fulminating. The mortality among untreated cases or cases that were treated too late is remarkably high and amounts to about 70%.(138a). This corresponds somewhat to the findings made during the last year in Germany (see table 10). Other cases begin subacutely, slowly, persist without fever, and are at first difficult to classify symptomatologically.

In the clinical investigation there are, besides the local findings (sore throat, pharyngitis, purulent rhinitis, conjunctivitis, otitis media, etc.) the indications of meningeal irritation (stiff neck, opisthotonus, positive Kernig and Brudzinski signs, weakening or increase in reflexes, hyperesthesia, photophobia, muscle twitches)

and encephalitic symptoms (vertigo, vomiting, visual disturbances, paralyses, most severe headaches, mental disturbances, etc.) predominate. Ptosis, myosis, and pupil rigidity are sometimes observed (163).

In the peripheral blood there is regularly a definite leukocytosis with general granulocytosis, commonly in the earlier stages one also finds a monocytosis or a picture corresponding to that of a listeriosis (227,242b,319 and others) and in later stages of the disease the monocytosis again disappears.

The spinal fluid pressure is increased. The protein reactions are generally strongly positive; the sugar content sharply lowered, in a few instances however it may be increased. The appearance of the fluid is, because of increases in cell content, usually cloudy or purulent but sometimes, however, it is clear. Among the cells the neutrophil granulocytes generally predominate, afterwards there appear, in increasing proportion and many times overwhelmingly, monocyte-like cells with single nuclei, as well as lymphocytes. The monocytes are one of the most important indications in the spinal fluid in Listeria-meningitis. In smear preparations one finds extra- and intra-cellular short gram-positive bacilli, that may be confused with pneumococci, streptococci, and/or Corynebacteria.

If one preserves these kinds of spinal fluids for a few hours at room temperatures or in an incubator, multiplication of the bacteria

is often rapid, and the visual field in smear preparations is strewn with countless bacteria.

Figure 20. Spinal fluid smear in Listeria-meningitis.

In other cases the number of bacteria is so small that only after many days of incubation is the detection of bacteria in the cultures concerned successful.

From the fact that the cases reported on lately have more often been diagnosed early it must be concluded that the microorganisms were many times not recognized or were taken for contaminants.

Perhaps many Listeria infections are in reality hidden among the so-called therapy-resistant pneumococci, meningitis and other inflammations of the meninges from Diphtheria-bacteria, Corynebacteria Acidobacteria, and so forth. Finally, the listerioses of the CNS that are connected with psychotic phenomena should not remain unmentioned (151,268).

In three cases of this kind which to be sure were only serologically verified, reported by Timofeeva and others, a chronically remitting course (6 months to 1 1/2 years) was observed consisting of varying fever, with depression, sensory illusions, delusions, obtrusive actions, aggressiveness with the appearance of hallucinations, aberrations, and so forth. Neurologically was found atypical increased reflexes, anisocoria, vestibular damage, unrestrained laughter, muscle twitches,

hyperesthesias, and so forth. With only a slight increase in the cell count and an increase in protein content of up to 0.99 mg. % there appeared in the spinal fluid pathologic colloid curves of a paralytic or a luetic type.

A few patients survive the acute stages of the disease,--also with conservative treatment--and a chronic meningitis results, that can persist for many weeks and months, and must be distinguished from clinically similar processes by differential diagnosis. The cell increase then frequently expresses itself as a disproportionate amount of lymphocytic and monocytoid cells, so that the existence of a tuberculous disease may be simulated (32).

The spinal fluid cultures remain Listeria-positive up to the time of cure, Schulz and coworkers were able to culture the bacteria from the spinal fluid of an adult even 120 days after the beginning of the illness. Jaeger and Myers isolated Listeria from the blood of a patient 42 days after the beginning of the illness, although they were able to cure the meningitis, clinically, with penicillin; the blood culture was first negative after 72 days.

Besides, in a case of tuberculous meningitis a listeriosis meningitis may also be present, as was demonstrated by Boese and Worth.

The healing of listeriosis of the CNS is not very seldom

connected with persisting damage. This kind of defective cure manifests itself in small children by the development of a hydrocephalus (156a,274) besides ptosis or strabismus (270) visual disturbances, etc.; in adults as speech defects, loss of memory, limitations in movement, nerve paralysis, ptosis etc. (70,232). Very little is known today about the sequelae of Listeria-infection in the brain and meninges. Meanwhile it is to be noted that serologic tests in persons with postencephalitic symptoms and brain damage not seldom yield titer values that are compatible with the assumption of a persisting Listeria infection (151,242j,312,318).

As an intrauterine infection with Listeria may even occur the possibility should not be excluded that in cases of brain damage at birth, which are grouped together, under the collective classification of encephalitis congenita (Virchow), in one case or another may be the result of surviving listeriosis.

The provocative nature of Listeria in meningitis and encephalitis is uncontestable, because the bacterioscopic and cultural detection in connection with the clinical picture forms an indubitable etiologic clarification.

The pathogenesis is certainly not unique. In relation to the septic general infection the possibilities of traumatic origins must also be investigated. Russian authors (cited after Bilibin) speak of

a post-traumatic Listeria meningitis. Hematologic dissemination was proved in a number of cases by positive blood cultures, and autopsic findings, and a dissemination probably was made in other patients by the finding of Listeria--infected foci in the vicinity of the meninges (conjunctivitis, sinusitis, septic sore throat, mastoiditis, and so forth).

It is probably combined also in double infections with viruses (242h) and protozoa, for example, Toxoplasma (242k,312). There are a few reports on mixed infections with other bacteria, for example, in which, Pneumococcus mucosus, Streptococci, etc., were isolated from the spinal fluid in cases of Listeria-encephalomeningitis. These findings do not indicate a special neurotropism for Listeria, but rather for the presence of other noxa, that are made possible by different bacteria, that lodge themselves in the meninges. It must still be determined how great a role is played by virus infections in the sense of a pathway of infection in case of listeriosis, for example.

e) Septic granulomatous course-forms (Granulomatosis septica seu infantiseptica). In the same way as in the animal kingdom the generalized Listeria-infection in humans results in typical focal phenomena in the organs, that are characterized by the formation of Listeria-granulomas. According to the location of these foci the various clinical symptoms appear, of which some have already been

discussed in the previous section.

Just as in the resection of patients with listeriosis of the CNS are commonly found widespread liver, spleen and lymph node involvement, it is assumed that the favorable cases, which occurwith the picture of a listeriosis and so forth, are based on similar pathologic anatomic involvements.

The transition between the individual syndrome forms are fleeting. The clinical picture is determined by the particular organic involvement that predominates.

Among the Listeria-types there is no special type that gives rise exclusively to a septic granulomatosis, be it in newborns, infants or small children or be it in adults. Serologic investigations (242j) have clearly demonstrated that serotype 1 as well as serotype 4 can cause the same type of disease. The delineation of a special type (204,210b) is not justifiable (242f,157c,191,317 and others), and has meanwhile been abandoned by Potel (205).

The first observations of a septic granulomatosis in newborns and infants, and later also in adults, originated from Burn in the USA (1933/34). It is to the everlasting credit of Reisz, Potel, and Krebs, as well as the systematic studies of Potel and his coworkers (204) to have found, on reexamination of this picture of the disease, one of the most important course-forms of listeriosis.

In 1951 Reisz, Potel, and Krebs reported the results of their studies on 20 instances of stillborn and prematurely born babies, and infants, that had died of an infectious disease caused by bacteria. Pathologically-anatomically it was a question of a disease syndrome, that was known to the pathologists a long time ago under the heading of argyrophilic sepsis, pseudotuberculosis, miliary liver nodules and so forth but of which the etiology could not be, up to the present time, explained. Potel succeeded in finding the individual cause of this disease by the detection of a bacterium that he named Corynebacterium infantisepticum.

This bacterium was identified by Seeliger (1951) as Listeria monocytogenes. Thereupon it was proved that in the case of granulomatosis infantiseptica it was a question of a Listeria-infection. In a large portion of the patients described in pathological-anatomical publications (compare table 9) the designation of listeriosis might have been applicable.

The significance of the labors of Potel lies above all else in that granulomatosis infantiseptica remained up to that time an almost unknown, infectious source of fetal death, abortion, premature births and fatal cases during the first stages of life, which he has explained. The number of cases in the region around Halle alone already totals 48 (323) and in numerous other places in Germany further

reported cases could be bacteriologically or serologically confirmed (table 10). Independent of the German authors were the same course-forms reported in 25 fatal cases in Czechoslovakia (191,322) and lately are also known in Canada and England (299,317).

Apparently listeriosis is, together with erythroblastosis, syphilis, and toxoplasmosis, one of the most frequent causes of stillbirths and fetal injury.

The following reports on the clinical aspects of this disease picture are based on 42 case histories which Potel has obligingly placed at our disposal, besides the reports of a great number of other authors (1,40,41,63,94,95,157c,156,191,204,249,254,279,316 and others) as well as our own observations.

The disease is not clinically detectable in the fetus. Severe damage to the fetus is first manifested by cessation of movements of the child and by stoppage of the heart beat. These indications lead generally to premature- or still-birth.

Potel determined that the earliest embryonic month for the disease to take hold was the fifth month of pregnancy.

Unpublished reports of the author that were formed from results of investigations of the mothers serum after abortion (also after artificial abortions) show however that fetal damage by Listeria-infection is also to be considered earlier in the stages of pregnancy.

If the infection first occurs at a late stage of pregnancy, dead fetuses may be born just as well as living children. Those born alive often appear to be already diseased, many others however seem to take a new lease on life. Many prematurely born infants die only a few minutes after birth, the majority die in the first and second week of life.

The clinical phenomena are not characteristic. They are considered by the doctor and the midwife as debilities, amniotic fluid aspiration, nutritional deficiencies, and so forth. The source of infection is not to be determined clinically.

Often the doctor finds a newborn that is moribund in appearance which does not survive the morning after delivery in the hospital (63).

In the foreground stand injuries to respiration and circulation of the blood, with dyspnea, breathing by movements of the nasal alae (stertorous breathing?) transitory or fatal episodes of apnea, chilling, cyanosis up to turning veritably blue, vomiting, episodes of convulsions, low moaning, premature passing of meconium (sometimes even in the amniotic fluid), passage of slimy stools. Diarrhea and the usual appearance of roseola or papules belong also to the clinical picture. Turgor is increased, the muscle tone on the contrary is lowered. If the children survive the first day, a purulent meningitis also appears, then, which seals their doom.

In a week old infant the disease often begins as grippe-like disease, cough, catarrh, dyspepsia, and bronchopneumonia.

Since the child is generally very severely ill and the attention of the doctor is given fully to means of preserving its life, only often a little, or even no reasonable investigations are made, so that the underlying causes are hereupon still not fully understood.

Taking X-rays of the lungs reveal in a few patients some small not sharply defined, bronchopneumonic floccular shadows. By auscultation and percussion only occasional rales and transitory differences in reverberations may be detected.

The liver and spleen are only very rarely found to be enlarged on palpation and to have a soft consistency, but sometimes a normal consistency. On account of the development of icterus neonatorum, that may reach a point (due to the effects of the disease) of a definitely yellow appearance, serologic tests for the differential diagnosis of the other liver diseases are of no value here. Also

Figure 21. X-ray taken of the lungs of a new born infant, with listeriosis (side view) (Potel).

if the inspection of the rear wall of the throat in newborns is difficult, it should never be overlooked in case listeriosis is suspected, since in that case we regularly find the characteristic multiple granulomata on the posterior pharyngeal wall (210a,b).

The temperature curve is not characteristic. In some cases a highly febrile course is observed, in others a hypothermia.

Concerning the heart besides a tachycardia there are no special findings to be made. The common final circulatory failure is in the majority of cases definitely of central origin. Erdmann and Potel suspect an indirect influence upon the circulation as a result of the affliction of the adrenals.

The blood count shows a more or less definite leukocytosis with displacement to the left, and the appearance of immature red and white cells. Monocytosis was sometimes observed, but was missing in other instances.

An etiologic diagnosis is not to be made clinically, since symptoms such as apneic episodes, circulatory failures, impairments of ability to drink (swallow) and so forth are common indications of prematurity. In differential diagnosis birth injuries such as tentorial tears must come into consideration, with their resultant widespread bleeding, which may mask a Listeria meningitis; and congenital syphilis may take a very similar course.

The pathological-anatomical findings are on the contrary quite characteristic and are distinguished by the presence of miliary nodules in the organs (for examples, see p. 89 ff). In the early stages however, macroscopic changes may be entirely lacking.

Therefore the establishment of a definite diagnosis is assured only by bacteriologic methods. For basic investigations, the meconium, the organs, particularly the brain and spinal fluid, and also the placenta and lochia should never be overlooked (see p. 109). Retrospectively serologic investigation of the maternal blood may yield valuable findings.

The infection of the fetus may occur by different pathways. It is fairly certain that it does not occur solely by way of the blood vessels (diaplacentarily). Many people have shown numerous times that commonly the amniotic fluid is first infected intrauterinely and the microorganisms are absorbed through physiologic swallowing of the amniotic fluid. The infection of the amniotic fluid conceivably takes place via external as well as via internal (diaplacentary) routes.

Infected amniotic fluid, that not seldom contains meconium, has a muddy or cloudy appearance and is colored greenish to brown.

Infections may also occur during birth from aspiration of amniotic fluid containing Listeria with resultant bronchopneumonia and subsequent generalized infection.

The infection that occurs intrauterinely or intra partum is not always acutely fatal. This may be demonstrated by observations on the birth of twins.

Indeed, many times the death of both twins is reported. In

other such instances (table 10; 238) one twin, however, survives. If consequently it is not to be concluded definitely that in such cases the infection of the surviving partner generally was not proved, this assumption has also little validity.

Possibly the infection does not always run acutely, but remains latent in a few cases or takes a chronic course.

One such case was reported from Greifswald (94b,95). While one twin died immediately post partum from listeriosis, the other died three weeks later from interstitial plasma cell pneumonia. The beginnings of the formation of Listeria-meningitis was stopped by aureomycin. Resection revealed a healing, septic, listeriosis. With the exception of the foci in the liver, all remaining foci were practically healed.

It is still not clear whether survival of the infection during the embryonic stage is possible and results in a defective healing as in the case of the roseola or toxoplasma infections.

Finally it has been demonstrated that these forms of listeriosis that appear relatively often in newborns also appear at other ages of life.

In the review of Oebike the ages of--according to the opinion of the authors--infants dying of listeriosis were given as 1 to 420 days (about half of the 22 collected cases were 2 months or more old); 4 fatal cases in infants that were reported by Schmitz ranged in ages from 1-4 months.

Some newborns developed normally and became ill later. The definite intrauterine or intravaginal infection in the cases of the stillborns or the ones born ill, that was generally believed probable in the case of the newborns that became ill immediately post-partum, may be considered to have occurred in these cases only if one supposes a long latent period or a slow tortuous course. However, since a peroral transmission of the microorganism is to be considered at all times, the infection in such cases might occur besides outside the uterus.

This pathway was confirmed in granulomatous listeriosis patients in the cases of adults, as was described by Burn in a 53 year old adult patient with otitis media, meningitis and sepsis as well as by Seeliger (242g,133) in a 50 year old shoemaker in Malmedy with septic granulomatosis without involvement of the CNS.

The latter became ill on Christmas, 1950, with noncharacteristic symptoms such as tiredness, weakness, and loss of appetite. After 4 weeks a swelling of the abdomen was noticed, which was due to dropsy. About the heart was found a pericardial effusion; also in the areas of both lung fields massive pleural and intralobar effusions on both sides could be detected either clinically or roentgenologically. The liver was hard, smooth, and extended beyond the edge of the ribs in the medioclavicular line about three to four fingerbreadths. Repeated

bacteriologic tests of the pleural exudate, ascites fluid, and blood yielded no results. The BKS (precipitation rate of cells?) was not raised, the blood count, except for a mild anemia was normal. The liver function tests showed: bilirubin index 1.16; Takata Ara- test negative; Weltmann 7; Gross' test 0.44 to 1.54; bilirubin direct test 3.03; cadmium sulfate test negative. Electrophoretic protein analysis showed considerable deviation from the normal content: albumin 46%, alpha globulin 29%, beta globulin 18.6%, gamma globulin 6.4%.

The patient appeared to have the symptoms of a progressive atrophy of the liver, and after about a half a year of the disease went into a fatal hepatic coma. The clinical diagnosis favored hepatosplenomegaly and polyserositis.

Only through the bacteriologic investigation of the organs, occasioned by almost overwhelming findings in the liver, spleen and lymph nodes, that were not easily cleared up, was it possible to explain the disease syndrome etiologically as definitely a malignant listeriosis. The case holds interest, when one realizes, that such a bacterial disease lasting for months could not be diagnosed intra vitam despite repeated bacteriologic observations.

The appearance of widespread effusions appears also in certain listeriosis forms in adults to be pathognomonic. Therefore people will have to think more than they do now of listeriosis in cases of

polyserositis, above all if the exudate quickly gels and is rich in phosphates.

f) Listeriosis in pregnancy. The hypothesis of an intrauterine or intravaginal transmission to the fetus or to the newborn presumes an infection of the gravida. Its detection and its understanding are of outstanding significance for a workable prophylaxis.

While in the reports of American writers no important facts concerning a previously existing maternal disease in cases of listeriosis in the early period of childhood are to be found, there are other reports concerning exactly this happening, for example in a case published by Slooff:

A ten day old infant became ill with a bacteriologically verified Listeria meningitis. The mother had conjunctivitis and rhinitis with bloody secretions during the last month of pregnancy. A few days post partum she died of meningitis. Although gram-positive bacilli were detected in the spinal fluid, bacterioscopically, the cultures remained sterile perhaps because of the chemotherapy administered. The child on the other hand they were able to save. We could hardly go wrong in assuming a direct connection between the two patients.

Listeria meningitis in pregnancy has also been observed in Germany (242h).

A 26 year old woman became ill suddenly in the seventh month of pregnancy with paralysis of deglutition, and pain in the head and limbs. The temperature rose on the second day of illness to 39.5°C. along with which she manifested clinical signs of meningitis. In purulent spinal fluid, (compare Figure 20) Listeria were detected in pure cultures carried out twice.

On the contrary numerous publications on behalf of the pathologists say practically nothing about maternal disease in cases of stillbirths, premature births, or newborn babies ill with the so-called pseudotuberculosis, that--as already said--is frequently represented as nothing but another form of listeriosis. An infectious source of miliary nodules in the liver will generally be regarded with skepticism.

Moreover, Patocka and coworkers report that the majority of the mothers of newborns ill with listeriosis presented themselves no disease during pregnancy that people could define as listeriosis. In a few cases however mild "grippe-like" infections will be noted. The authors conclude that the infection generally runs a subclinical or asymptomatic course and is only revealed by the illness of the offspring.

On the contrary other authors (1,157a,210b,254) were able to raise a series of histories, which showed clues to the course of

listeriosis in pregnancy.

Many gravidae became ill specifically a few weeks or days before the abortion or delivery, with acute febrile states with headaches and chills, others experienced an angina. Diarrheas were sometimes reported, pyelitis repeatedly appeared.

The white blood cell count was as a rule unremarkable, apart from in a few instances leukocytosis with increase of segmented cells and with deviations to the left as well as the usual monocytoses.

In a few cases occurred during the course of the illness a puerperal fever that was generally without complications. The temperature generally subsided after confinement, a few patients however also had fever that was generally without complications. The temperature generally subsided after confinement, a few patients however also had fever during the childbed period.

Therefore the acute, febrile, grippe-like, generalized infection of the maternal body is characteristic of the mild cases. Whether also many cases of pyelitis gravidarum are due to listeriosis cannot be stated with certainty, as the detection of the bacilli from the urine has to date seldom been successful immediately post partum.

Because it is truly a question as to the presence of a Listeria infection in cases with these symptoms that are less characteristic, they must be interpreted on the basis of the results of bacteriologic and serologic studies.

The microorganism has been successfully cultured from the blood during the childbed period; it was sometimes detected in the lochia, in vaginal scrapings, in the urine and in the placenta (68,191,204,316,317). It should be noted here that the detection of the microorganism is made particularly difficult because of the bacterial flora of the lochia.

The identity of the microorganisms isolated from mother and child was proved biochemically and seroanalytically (204,242j).

Not yet definitely explained is the long interval of time between the maternal infection and the outbreak of disease in the fetus. In general this interval usually appears to be relatively too long for diaplacentary bacterial infections. In the case of listeriosis it is however, repeatedly reported that the premature or stillbirth follows immediately after an acute febrile infection of the gravida with chills. Hegemann and Simon put forth the question as to whether this is not a result of fetal infection long after the maternal infection took place. If that proves correct, the Listeria infection in utero would not commonly be distinguished from other bacterial infections.

Unfortunately the clinically little apparent Listeria infections are not always bacteriologically detected during pregnancy. Because they often have a chronic or subacute course, the body of the gravida has the ability to form humoral antibodies, that are serologically detectable.

Listeria agglutinins are found also, however, in healthy persons, whereby a positive Widal reaction that must be conducted with the antigens of the different types, is not always capable of proof. According to Seeliger only titers in a serum dilution of 1:302 or more or clear rises in titer of at least two stages are of diagnostic significance.

The complement fixation test is more specific, the propitious use of which, in listeriosis in pregnancy, Seeliger first reported (242c).

The results are comparable to those of Patocka, who worked out a similar method independently. Examples may be found on p. 124 ff).

Both tests complement each other from opposite points. In high agglutination titers the complement fixation test may be negative; the reverse has not been observed and may hardly, thus, need to be considered. A positive complement fixation test in serum dilutions of 1:10 and over is to be considered a sign of a persisting or generalized infection. After healing it will quickly become negative or sink to a low value.

They have repeatedly succeeded in revealing, by the use of these serologic methods, the presence of a clinical asymptomatic listeriosis in pregnancy or to yield findings that are compatible with the diagnosis of a listeriosis. These women have as a rule already experienced abortions or stillbirths and therefore have consulted the clinic (hospital).

(242,307,311 et al.).

The individual titers were in these cases generally remarkably high, and the complement fixation test yielded in the presence of Listeria antigens strongly positive results in dilutions of 1:10 to sometimes 1:80. These titers remained constant, with small variations, without treatment but diminished as a rule however, after therapy with sulfonamides and antibiotics.

The observations to date lead one to conclude that it is possible through the use of chemotherapy or administration of antibiotic preparations to overcome this kind of infection and to further an undamaged course of the gravidity.

One of the chief difficulties of serodiagnostic methods lies in that from the titers it cannot be conclusively ascertained whether an infection still exists at the time of the testing. This is quite probable in case of positive results of the complement fixation test at high dilutions. The search for the microorganism should then be performed under any circumstances.

Pertinent serodiagnostic tests are promising and should be performed in all cases of unexplicable febrile diseases in pregnancy, especially in pyelitis, and the same after abortion of stillbirths and also however in case of renewed pregnancy subsequent to a previous abortion, etc. This goes particularly for pregnancies among farm

folks, and in women who directly or indirectly handle animals and their products.

It has not been shown that the microorganism after survival of a general infection, remains in the vagina and may damage the fetus in cases of renewed pregnancy. In a series of verified listeriosis cases, many stillbirths were anamnestically revealed.

It is not known whether an infection may occur by way of the mother's milk.

Findings in the animal kingdom as well as the results of the research of Potel in pregnant guinea pigs with positive bacterial findings in the mother's milk makes it probable that the thing behaves perhaps similarly in humans.

To date there is nothing to say definitely about the morbidity of listeriosis during pregnancy. It appears not to be slight. In the middle of Germany (Halle/S) during a period of 3 years among 5,000 births 170 suspected cases were investigated serologically and bacteriologically, whereby six were proved to have listeriosis (1b). The results of specific research in the region around Bonn yielded a still higher quote of suspected cases (242j). Exact figures will be published shortly by Bruenig and Fritzsche from Leipzig; in a period of 10 months the perinatal death rate among 3,246 clinically confined

children was 5.42%, then proved listeriosis mortality was 0.154%. By immediate detection and treatment of these kind of cases the perinatal mortality may therewith be further lowered.

g). Other forms of listeriosis: The total symptomatology of listeriosis is definitely not finished with the pictures of the disease set forth here. Just as in brucellosis we must reckon with a total clinical syndrome. Only lately has Wenkebach in Argentina found that Listeria in males can cause a protracted urethritis.

His observations consist to date of five reported cases, sometimes mixed with gonorrhea, in which a common source of infection or promiscuity cannot be definitely excluded.

Thereby it might be concluded that transmission with all subsequent results may possibly system from cohabitation also. It is to be determined also whether this form of Listeria infection also plays a role in listeriosis in pregnancy, or whether it appears in noteworthy numbers among people who work with animals.

Finally there are still the fully symptomless cases of listeriosis to consider. An instructive example of this is found in an observation of Myers (319) on a clinically healthy young man, in whom during a routine chest x-ray suspicious foci were found in the lungs.

There was neither fever nor blood changes nor obvious symptoms.

To exclude brucellosis and tuberculosis suitable cultural investigations were conducted. While the blood cultures were always negative, L. monocytogenes was twice successfully isolated from aspirated bone marrow.

Further atypical course forms were reported by Hoffmann and Walbrück, for example in a young woman with severe anemia and endocarditis, from whose blood Listeria were cultured. Healing with a residual valve injury resulted after intensive spinal therapy. Likewise Listeria positive was the blood of a 47 year old man, who died of a bronchial carcinoma with widespread metastases that were detected by a pathologist.

3. Pathologic anatomy.

As already stated, the pathologic anatomic picture of granulomatous listeriosis has already been known for a long time under the classifications of "pseudotuberculosis" "argyrophil sepsis" or miliary and organic necroses (table 9) (182,204,210,228,224a).

The designation made by Eberth (1885) for a disease of rodents--"pseudotuberculosis"--was carried over into human pathology by Henle and since then has been used by many researchers. Many unexplained things remain here, therefore one would do better to avoid this classification, which leads easily to false interpretations.

In Germany the organic changes in human listeriosis have been

discussed in a number of publications in which it is allowed that many earlier cases were without doubt classified under other names (94a,b, 133,210,228).

The following summary gives the essentials of the sources quoted, of which for study the original articles must be consulted by individuals.

Listeriosis in humans is characterized by the appearance of miliary nodes in the organs. The macroscopic changes correspond largely to those in the animal kingdom in experimental infection and in diseases occurring under biologic conditions (in their native habitat).

The frequency of organic involvement varies from case to case. Appearance and thickness of the formation of foci are different and vary according to the mode of infection, infection-dose, and age.

The disease among newborns and infants is distinguished by a disseminating involvement of numerous organs with pinpoint to millet seed-sized nodules.

Almost always a widespread involvement of the liver predominates, which, similarly as in miliary tuberculosis, is permeated with grayish yellow nodules. To these correspond also the findings in the spleen, adrenals, lungs, especially the subpleural areas, esophageal mucosa, posterior wall of the throat, and tonsils. Nodules lying subepithelially often break through, so that necrotic-ulcerating foci are formed (for example, on the tonsils). Generally one finds definite bronchopneumonia

with disseminated, submiliary necroses of the alveolar stroma, besides purulent otitis media, kidney necroses, and so forth. Also these foci may be found in lymph nodes, thymus, bone marrow, heart muscle, testicles, and muscles.

The intestinal tract is affected in varying degrees. Nodules appear mostly in the region of lymphatic tissue in the small intestine and appendix, and were also here in earlier observations only seldom missed. The colon and the rectum are not as a rule affected; although at the same time a necrotizing focal or imbedded, disseminating colitis with concomitant fibrinous peritonitis were repeatedly found. Stomach and duodenum presented themselves many times as free.

In most disseminated cases the soft brain coverings reflected the miliary spread clearly (see also further below).

An involvement of the skin not previously reported was described by Reiss. This involved mainly the back and lumbar region, and to a lesser degree the skin of the extremities. It consisted of the appearance of millet seed- to pinpoint-sized gray white, raised papules which are surrounded by a red border. These changes are not so clearly seen during life (204e).

In the few cases of listeriosis in adults that were autopsied, findings were revealed that were similar in principle but sometimes, however, divergent. Thus Schamsow found in a 35 year old man, besides

ulcers on the left tonsil, nasal septum, and oral mucosa a necrotizing abscess forming focal pneumonia, purulent necrotizing bronchitis, and periportal (around the portal vein) granulomas of the liver. In spots there is a resemblance to an agranulocytosis.--In the case described by Jung and Seeliger severe changes in the liver and spleen predominate, which resemble macroscopically an infiltrative nodelike tumor.

In the liver was found focal areas of increasing involvement with clay-colored aspect and irregular, map-like boundaries, in between grayish red areas with numerous small, gray white nodules (Figure 22).

The splanchnic nodules were walnut- to hazelnut-sized, one reached the size of a small hens egg. Also the mesenteric lymphnodes were enlarged and infected with nodule-like foci. The lungs were likewise attacked, the CNS on the contrary was free from infection.

In Listeria encephalitis macroscopic changes may be totally absent. Often one finds however in submiliary encephalomeningitis submiliary nodules up to miliary sized, in the region of the leptomeninx as well as at the base of the brain and over the convexity, mostly about the larger blood vessels. The brain tissue itself is hyperemic, rich in fluid, and sometimes affected by diapedetic bleeding. In the spinal cord tissues one may find in various quantities pale yellow focal softenings that are about the size of a grain of rice. The choroid plexus is hyperemic to a great degree. In purulent meningitis

the subarachnoid spaces are filled with a thick purulent exudate.

The histopathologic appearance of human listeriosis shows no basic differences from the animal tissues, by way of reactions. The histologic appearance of the miliary nodules shows the same characteristics. The different states of nodules in the process of formation jumbled up together make in the same organ an often very distinct picture.

Figure 22. Liver of an adult who died of listeriosis (242g).

According to Reiss the appearance of the foci is not connected with any particular site on the liver lobes. The focus-like areas are fairly sharply set off from the healthy tissue. The action of the microorganism leads immediately to a primary necrosis with considerable increase in reticuloendothelial elements (cells) (Kupffer's star shaped-cells, histiocytes, epithelial cells and monocytes) so that a granuloma is formed. The interior will be mostly necrotic, and in the center there is a white non-homogenous coagulation necrosis (94a) which spreads itself from around the nodule into the periphery. The border zones are filled with more or less numerous inflammation cells. From them capillary and histiogenic resorption cells spread outwards, between which the cell and nuclear fragments lie. Purulent effusions and caseations do not, on the contrary, belong in the picture of Listeria granulomas.

In the neighborhood of the central necrosis the microorganisms are found in considerable numbers, and uncharacteristic striations, that are best made evident by Gram staining or by silver staining with the method of Levaditi.

One may correspondingly differentiate initial foci, proliferative, and resorptive granulomata, which many times appear adjacent to one another.

Similar changes irrespective of what age they appear, are found in all affected organs. Sometimes stationary overgrowths of cells predominate, other times changes into lympho-histiocytic infiltrations are detectable. Frequently, vessel necroses andcell-like intima granulomata are to be found as a sequel to septic dissemination.

In listeriosis of the newborn the placenta shows generally a picture of an intervillous inflammation (210), Hagemann and Simon found in the region of the maternal decidua and chorionic villi considerable concentrations of the microorganism. On the contrary the bacteria content of the chorion lamella, was extraordinarily sparse. Correspondingly the foci in the liver and kidneys often manifested a high degree of cell proliferation. In the area of the chorion lamella individually reactive epithelizations and circumscribed necroses; a wider, cell wall formed from great monocytic-histiocytic-resorption cells was found by Hagemann and Simon at this site to have particularly

good defensive functions. The authors named supposed that the basal membrane formed a definite barrier to the transmission of the micro-organisms from the maternal to the fetal circulation.

The tissue changes in listeriosis of the CNS are likewise distinguished by granuloma formation.

Small rice grain-like foci appear often in contact with a radiating pial vessel particularly in the upper and middle cortical strata. Such cerebral cortical granulomata were not seldom manifested by discus-like infiltrations of the leptomeninx (Simon). In the vicinity of these foci it amounted to total destruction of the tissues with phagocytosis of the nuclear and fibrinous fragments by histiocytes. At the periphery no glial infiltration is generally detectable. Also in the presence of ulcerating foci in the spinal cord tissue glial reaction is often absent. Frequently one finds perivascular accumulations of lymphocytes. In the region of the perivascular tissues thick polymorphonuclear cell wall is formed. The granulomata lying thickly under the ependyma project into the ventricles and considerably narrow the outlets. On account of the usually severe involvement the plexus choroidei with Listeria forms definite cell infiltrates (mostly with few leucocytes) and granuloma-like concentrations. The spinal fluid is rich in cells (predominantly mononuclear cells, leukocyte components quite discernible) and contains the microorganism.

The pathogenesis of the newborn and infant-listeriosis, as the course form best investigated to date, in human listeriosis, may be reconstructed from the study of the histopathologic changes.

Reiss gives the following pattern for the pathway of infection and the spread of the infection in the immature organism:

Infection of the mother
Diaplacentary infection of the fetus via the umbilical vein.
Septicemia of the fetus with changes in all organs.
Excretion of bacteria with the urine into the amniotic fluid.
Infection of the amniotic fluid.
Aspiration and imbibing of infected amniotic fluid.
(Changes in the respiratory system; otitis media in some cases).
(Changes in the digestive tract)
Possibly bronchopneumonia or enterogenic septicemia.

Many of these appear here only hypothetically, for example the assumption that there is repeated infection first diaplacentary and then per orally. --Schmitz reports on "miliary liver necroses": "The disease as a whole appears therefore often as a congenital, overwhelmingly enterogenous (amniotic fluid) less often as an aspirative-pulmogenic, and still more seldom as a diaplacentarily (?) transmitted generalized infection of a septic-metastatic nature, that usually makes its

appearance as one of the diseases of the digestive tract. It spreads from there as the microorganisms overwhelm the liver immediately via the portal vein, that is thus the organ most often affected, and may from there like any infection, conquer the lungs and ultimately all organs of the greater circulatory pathways."

On the contrary there are the findings of Hagemann and Simon, that largely discounted the possibility of a peroral, enteric infection as a sequel to an infiltration of the microorganism over the chorion lamella into the fetal amniotic fluid. These authors believe it more likely that the bacterial dissemination into the fetus occurs by way of the epithelium and capillaries of the chorionic villi.

The pathogenesis is probably not unique but varies with the age of life and the differences of the port of entry. The different views cited above are in no way contradictory to this. The findings consist of an heterogenous disease syndrome of immature as well as mature, premature and stillborn but sometimes occurring in infants. The latter need not have been unconditionally infected in the uterus or during birth. In general it is not very certain that in all the cases described by Schmitz it was a question of listeriosis, because the detection of the bacteria by bacteriologic methods was not successful.

4. Epidemiology.

Ever since comparative studies have shown that there are no differences in the Listeria strains isolated from men and from animals, and that all sero- and bio-types as well are present in man and animals too, human illnesses have been traced back to sources of infection in the animal kingdom.

This relationship that has been discussed many times may be however only relatively seldom verified today; because very often one cannot anamnestically find a definite focus or source of human infections. Generally it is discovered on painstaking investigations of this, that often the disease arose in the neighborhood of an epizootic or an enzootic, without however being able to determine the method of transmission in retrospect. Researches have only during the last decade yielded, from the bare hypotheses, a series of more demonstrable deductions. Thus a number of human infections resulted from dirt and filth infections as for example occurred in the conjunctival infections described by Felsenfeld in employees of a fowl market. A few of the cases found among humans in West Germany might be traced back to similar sources. Post-traumatic cases of listeriosis may also be explained thus. A dust infection is likewise possible. This applies above all to the cases observed in Russia, in which listeriosis appeared many times in neighborhoods or living quarters in which a mouse plague was rife. We may here

consider a transmission similar to that occurring in cases of tularemia (135). Besides also in cleaning of stables in which infected cattle were kept it may result in probable aerogenous infection, as the case reported by Ödegaard and coworkers of a fatal outcome of the disease in a Norwegian farmer, serves to teach us.

Usually the disease appears to originate from direct contact with infected domestic and laboratory animals, from the flesh of slain animals, animal cadavers, hides, game, etc. Also here one finds many parallels with tularemia. It is certainly no accident that listeriosis cases--as far as we have been able to ascertain to date--are found most often in persons, who had direct or indirect contact with animals. An increased danger of infection is definitely to be reckoned with in certain occupations.

The possibility of infection by foodstuffs is of considerable epidemiologic interest, because it can be considered as proved that in this way illnesses affecting groups and regular epidemics may be started.

It was shown already in 1917 by Atkinson in the observed increase in Australia of cases of the disease that was later identified as listeriosis, that the disease does not always have a sporadic nature in mankind. That may similarly apply in the case of Burn. The first

mass detections of listeriosis were accomplished in the region of Halle in 1949-1953 (204), in an epidemic in Jena (326) as well as in a not more closely defined area in Czechoslovakia (191).

In the overwhelming majority of the cases in Halle the use of raw milk was revealed as the source of the infection. Also, sour milk curds and whipping cream, were considered as possible transmitting agents.

Causal relationships between increases in cases of listeriosis among humans and corresponding cases among milk cattle had already been suspected since 1938 by Schmidt and Nyfeldt in a small outbreak in Denmark of a mild form of the disease. A few years later Wramby in Sweden was able to isolate Listeria from the udder of a cow ill with mastitis as well as from the milk itself. Potel had also found the microorganism in cows milk.

No doubt can thereafter exist that--in analogy to the transmission of brucellosis--listeriosis may also be transmitted to mankind by means of milk containing microorganisms. The logical conclusions this yields for the veterinary police are obvious (see page 62).

This is certainly not the only possibility for a per oral Listeria infection. Seeliger and Leineweber havecalled attention to probable links between listeriosis in fowls and human cases thereof. How the transmission takes place in this case is not known, and also, for example whether or not it could be done by infected eggs. In this state of affairs it is

also to be considered that Listeria-infected meat and game might succeed in causing an infection if used for human consumption. The classic example of per oral Listeria infections are cases of listeriosis in newborn, which result from the swallowing of amniotic fluid containing the bacteria.

The intestine of the newborn is extremely permeable and so, finally, it offers no resistance to the Listeria infection.

To what extent intermediate carriers and if necessary intermediate hosts play a role in the epidemiology of listeriosis is uncertain.

Also the possibility of a further spread by human sources of infection is not to be indicated offhand. More capable of being demonstrated are the transmissions of the disease during pregnancy intrauterinely or during birth to the fetus. An infection of the mothers milk is indeed still not definitely proved, but is quite plausible. The placenta and amniotic fluid of women ill with listeriosis contain the bacteria sometimes in large quantities, and also the lochia may be found to be infected with Listeria. Each case also demonstrates the danger that the transmission may result from the hands of the persons, for example, who actively take part in assisting at births. Because the amniotic fluid and placenta according to their appearance and smell often seem unaffected and also the newborn does not always immediately look sick, there may result further unintentional errors. It is hereby to be remembered

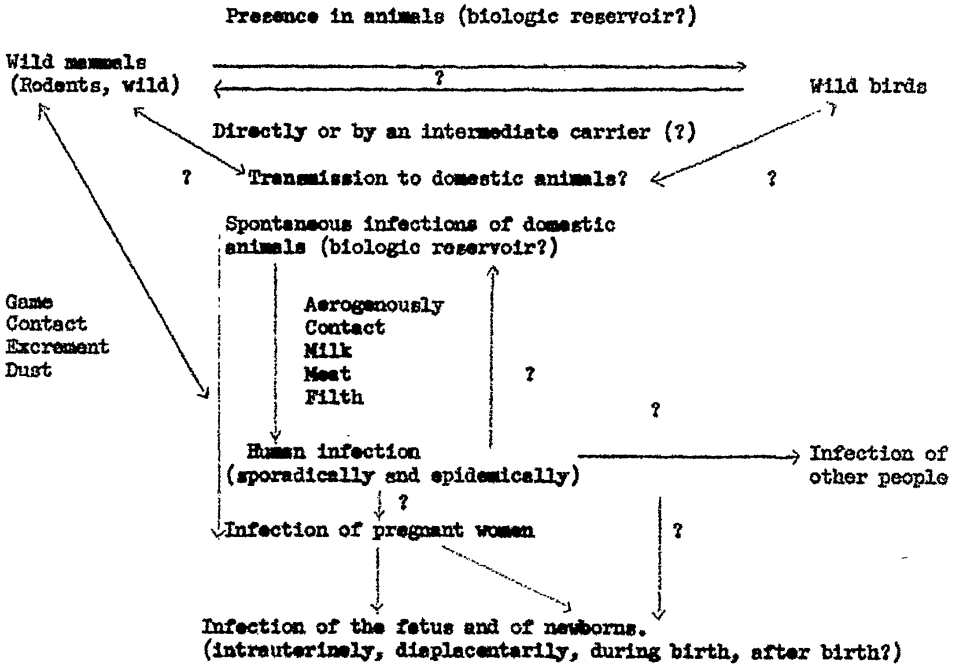
that listeriosis and the endangering of the newborn was generally unknown only a short time ago. Not less endangered are also the doctors and the nursing personnel as well as the relatives, as a number of sick and fatal cases teach us.

Finally it should be mentioned that Listeria can cause stubborn inflammations of the mucous membranes, and thus also to diseases of the genital tract. Probably infection and reinfection occur in this way among married people.

Nothing is known as to whether listeriosis can re-enter susceptible animals from human beings. If nevertheless no proof yet exists in this direction, it appears that this has not been excluded as a possibility.

The presence of all the Listeria-types found to date in animals in human infections is a certain indication of a multiplicity of sources of infection, which are to be found all over the animal kingdom. Until a short time ago the Type 3 strain found by Nyfeldt in humans made up the only exception. Afterwards on the basis of serological investigations this type presently became known as likewise the source of fatal ovine encephalitis (242j), so that there is also here no differences from the other types. Once again it should be emphasized that the serotypes show no differences pathogenetically or epidemiologically in either human beings or animals.

Schematic synopsis of the epizootiology and epidemiology of listeriosis.



In summing up it seems that the epidemiology of listeriosis shows an extraordinarily variegated and complex picture that in many instances must be still more closely investigated. In many respects it may be likewise compared to toxoplasmosis which has only been publicized during the past few years, as may also be seen from the far-reaching similarities

in the schematic description of both of these diseases (13,193).

In this simplified pattern the proven as well as the hypothetical extrinsic sources of infection (Doerr, Gotschlich) are clearly comprehensible. Intrinsic sources of infection (compare Habs) are still not to be formulated, because concerning immunity, carriers of bacteria, and properties of elimination no lucidity yet exists.

A connection between listeriosis cases and the time of the year is not perceptible. Indeed, Hahnefeld and Nisolk have shown thereof, that the cases of listeriosis in premature birth found by them appeared predominantly in the spring and summer (April to August); but also, numerous instances of the disease in the other months came to be known to the author, so that a connection with the seasons of the year is seemingly improbable.

5. Therapy.

As in the human medical literature up to the present time the listeriosis form that runs severely, particularly with CNS involvement, predominates, it might be concluded that the untreated disease is generally fatal. Kaplan in 1945 in a review figured the mortality to be about 70%; which also agreed with the findings in Germany. These kinds of reports are however deceptive, because they only concern the clinically manifest and bacteriologically proved cases. Today it is recognized that the infection often causes no clinical indications

to appear and relatively often runs a mild course and heals spontaneously.

The same is also being publicized in a few other infectious diseases that have been carefully investigated during the last decade, in which in the beginning we suspected a high mortality rate, for example, in the case of toxoplasmosis, or histoplasmosis, and of coccidioidomycosis.

In each of these diseases at first only the severe, many times fatal cases were known. Later studies then revealed generally a fairly wide distribution and a correspondingly smaller mortality rate than originally supposed.

A few course-forms of listeriosis make an exception; the disease in newborns and in adults with involvement of the CNS. Hereby the prognosis is generally unfavorable, at least doubtful. Notwithstanding the fact that such patients occasionally get well by spontaneous healing or with conservative therapy, it has been demonstrated that only the immediate administration of chemotherapeutic measures is capable of preventing the fatal outcome.

a) In vitro sensitivity of Listeria-strains:

Numerous native and foreign scientists have, generally in connection with the observation of pertinent cases, discussed the bacteriostatic or bactericidal action of chemotherapeutic agents and antibiotics on strains of Listeria. Insofar as it is a question

of experimental therapeutic research on animals here, he referred to the detailed statements, on pages 58 ff.

The statements on the in vitro sensitivity of the Listeria sometimes vary considerably from one another, so that it might be concluded that there are great differences in sensitivity among the individual strains. That is, however, not the case. Detectable differences do not originate from varying sensitivities of the microorganism, but almost always from the different methods of testing and of test procedures (157b).

The nature and preparation of the test substrate, the quantity inoculated, the age of the culture, the incubating temperature, and duration of incubation, influence the results of the research just as the test procedures in themselves (determination of the number of bacilli by the plate count, by turbidimetry, the test tube dilution test, the agar diffusion method, etc.). Besides, there are the differences in solubility, stability, concentration, pH range of optimum activity, and if necessary the rate of diffusion of the tested media as well as its diluent, that make it possible for the test results to vary, detectably or inapparently, under seemingly similar test conditions (42,52,64,116,157b,226 and others).

In the evaluation of these kinds of results one should never neglect the fact that in vitro environments under artificial test

condition in no way permit definite conclusions on the capacity for action in vivo. A drug that works excellently on a reagent glass may fail at the sickbed, because it is perhaps excreted too quickly or does not even reach the microorganism. This is particularly important in the case of bacteria that like the Listeria only relatively seldom and transitorily appear in the blood stream and the body fluids, but which however are therefore able to hide from the attacks of the drug for long periods of time by intracellular storage in the reticulo-endothelium.

Meanwhile the results of determinations of resistance in vitro allow the important declarations as to whether clinical activity may be considered at all. Bacteria that are found by the proper methods to be resistant in vitro against therapeutically attainable concentrations of the drug under consideration, do not behave otherwise in vivo.

In general, the drug acts in therapeutically feasible doses only bacteriostatically, and is bactericidal only in stronger concentrations.

Figure 23. Growth of L. monocytogenes under the influence of Supronal (157b).
Growth after 4 and 6 days at 22°C; 3, 2, and 1 day at 37°C; Supronal content expressed in mg%. (abscissa)
Unit of growth (ordinate) not given

Reports on the in vitro inhibiting values in the case of Listeria are found in a great number of publications (23,25,26,68,70,73,75,84, 87,96,106,123,171,178,181,188a,c, 204,241,242g,253,274,285,298,337,340, 341,346,348, and others.).

Linzenmeier and Seeliger (157b) found in the testing of 25 native and foreign strains of Listeria from widely different sources and of different ages under similar test conditions practically no differences in sensitivity against the individual drugs and was able to compute, after testing the strain by means of all the common test methods, the following average values for the complete inhibition of growth of L. monocytogenes:

Table 12. Growth inhibition of 25 strains of Listeria

Drug	Bacteriostasis by
Sulfonamide	6.25--12.5 mg. %, sometimes 25 mg. %.
Supronal	6.25--12.5 mg. %
Penicillin (1)	0.3--0.75 Oxford units per ml.
Streptomycin (1)	1.5--6.0 gamma per ml. (sometimes primary resistance or rises in resistance amounting to many hundred gamma/per ml.)
Aureomycin (2)	0.3--1.0 gamma per ml.
Terramycin (2)	0.6-1.25 gamma per ml.
Chloramphenicol (2)	1.0--3.0 gamma per ml.
Magnamycin (2)	0.18--0.76 gamma per ml.
Erythromycin (3)	0.037--0.15 gamma per ml.
Polymyxin B (2)	12.0--25.0 gamma per ml.
Bacitracin (2)	6.0--12.0 units per ml.

(1) Test material from the Grünenthal Company, Stolberg.
(2) Test material from the Pfizer Company, New York.
(3) Test material from the Schering Company, Berlin.

The only moderate sensitivity to penicillin, which was first noted by Foley and others, is not conditioned by intra- or extra-cellular penicillinases (345b).

With the proper research techniques which consider the special significance of the sulfonamide resistance determination in vitro (56,64,122,297) a definite inhibitory action of the sulfonamide on the growth of the L. monocytogenes is determined (157b,204). Reports reading otherwise are often the result of technical errors. It is interesting thereby that the sulfonamide activity is stronger at 37°C. than at 22°C., and their conclusive effect is detectable only after 3 days of observation. Plate tests with enumeration of the growing colonies are in the case of sulfonamide resistance determination, to be preferred over the test tube dilution tests (see Table 13).

Combinations of drugs were also tested for their action against Listeria. Thereby (157b) it was found that sulfonamide and/or supronal and penicillin have not only an additive, but also apotentiating (synergistic) effect. This may be clearly illustrated in three dimensional diagrams (Figure 24).

In bactericidal studies in vitro also was established a synergism between penicillin and streptomycin (Figure 25).

Table 13. Sulfonamide activity in vitro on L. monocytogenes at 22°C. (Inoculation of 0.3 ml. of a dilution of 1:1,000,000 per plate) (157b).

Reading after days	Number of colonies growing daily with sulfonamide concentrations in mg %						
	50	25	12.5	6.25	3.1	1.5	0
1	-	-	-	-	-	-	- (!)
2	-	12	45	50	65	50	68
3	32	54	20	2	-	-	-
6	25	-	-	-	-	-	-
8	-	-	-	-	-	-	-
Total colonies	57	66	65	52	65	50	68

Figure 24. Synergy of action of gantrisin and penicillin on L. monocytogenes (after 157b). (see page 99)

Figure 25. Bactericidal curve for L. monocytogenes with the action of penicillin, streptomycin, and a combination of both drugs, according to (157b). (see page 100)

b) Results of chemotherapy.

From critical evaluation of the in vitro inhibition values it may be concluded that drugs such as bacitracin and polymyxin B do not need to be considered in the question of the therapy of listeriosis.

Results with penicillin or streptomycin alone yield hardly any promise. This has also been demonstrated in practice. In a case of Listeria meningitis however a partial improvement was achieved, insofar as by the intramuscular and intralumbar administered penicillin therapy the meningitis was healed clinically, but the blood culture was still positive 42 days later (319).

With sulfonamides and sulfa supplementary combinations, as for example Supronalum, one may on the contrary expect better results and likewise with combinations of these drugs with penicillin.

The author and Leineweber observed after the administration of 1 million units of penicillin and 4 mg. of Supronalum per day the healing, after 4 days, of a severe Listeria meningitis case in the seventh month of pregnancy. On the contrary a case of a 15 year old youth remained without results after the administration of 7 gm. of sulfadiazine and 800,000 units of penicillin, but was later cured with terramycin (23). Also the combined use of penicillin (100,000 Oxford units every 6 hours intramuscularly) dihydrostreptomycin (30 mg. every 6 hours intramuscularly) chloromycetin (50 mg. every 6 hours) and sulfadiazine (0.11 g as an initial dose, followed by 0.06 g every 6 hours) in newborns remained without results, but were finally healed with the tetracyclines (156b). But even the highest doses of sulfonamides together with penicillin could not change the fatal course of a case that was brought in when moribund (163). On the contrary quick healing resulted in a 10-day old infant with Listeria

meningitis after the administration of penicillin (8 times 8,000 Oxford units daily over a period of 8 days) and sulfadiazine (600 mg. for 3 days, 1200 mg, 4 days). It was a question--as we later found--of a defective cure with the development of a hydrocephalus (274).

In individual instances it was difficult or almost impossible to say whether the healing resulted in fact from the action of the combination. For example in the last case mentioned the quantity of penicillin given is practically inactive in listeriosis, while the sulfadiazine dosage alone could have been sufficient.

Also the combination of penicillin with streptomycin was investigated.

By the use of both drugs (100,000 Oxford units of penicillin intralumberly, 300,000 Oxford units of penicillin intramuscularly (daily?) for 3 days and streptomycin intralumberly 25 mg (daily?) for 7 days plus 1 gm. in 4 doses daily intramuscularly for 10 days) Listeria-meningitis was successfully cured in a 2 1/2 year old child; in two other patients the treatment regimen failed because of primary streptomycin-resistance (241).

It is not out of the question that in a few of the cases quoted the treatment remained without results because the dosage was not high enough. This suspicion applies above all to the apparent failures of sulfonamides. Numerous clinical observations from the pre-antibiotic era (see table 13) have proved the value of sulfonamide therapy in human listeriosis. To their account is credited a definite

diminution of lethality of severe illnesses, especially listeriosis of the CNS.

No drug yields a therapeutic result if that drug is given too late, if the drug is unable to reach the microorganism, or if too small a dosage is used. Therefore sulfonamide therapy promises good results in listeriosis only if it is administered early and in large doses. In order to obtain a high blood level, the treatment is begun intravenously then in the manner of a combative therapy is given for many days per orally or intravenously in a similar fashion, whereby the dosage has been calculated according to body weight and general condition. In no case should the treatment be stopped too soon. If no improvement appears, at the latest, after 24 hours, it is hardly to be expected that the infection will respond to the preparation being administered. In such cases the addition of or subsequent use of antibiotics leads repeatedly to a cure.

In the chemotherapy of listeriosis the Listeria-active antibiotics of the tetracycline group (aureomycin, terramycin, tetracyn, and achromycin) should be designated as the drugs of choice because of their good compatibility, swift resorption, and their outstanding inhibitive activity against the bacilli that are to be reckoned with.

These drugs are generally given for many days per orally. The dosage regimen is calculated according to body weight and age. In adults the daily dosage amounts to 1-2 gm., divided into 4 to 8 individual doses at regular intervals in children correspondingly less.

A 15 year old Negro youth with neurolisteriosis was healed by the daily administration of 2 gm. of terramycin, after the other drugs (Table 13) had previously been found useless (23). --Aureomycin was active under the following dosage regimen in a 10-day-old infant: 100 mg. initial dose; followed by 50 mg. every 3 hours; after 6 days diminution of the dosage to half this quantity for further 14 days (156a). The preparation named above was also used, in this case (without visible effect). In a similar fashion (by 50 mg. chloromycetin, 50 mg. terramycin, and 50 mg. aureomycin, every 6 hours for 14 days, aureomycin for a further 8 days), a prematurely born infant with listeriosis was cured (156b). In mild subclinical cases during pregnancy and childhood 1-2 gm. aureomycin or terramycin for 3-5 days was sufficient for a cure (307,311,312). --Pohlmann and Boese recently reported on outstanding therapeutic results in the treatment of Listeria-meningitis in adults with terramycin (301,346).

Chloromycetin has also generally proved good. Thus a case of Listeria meningitis in an adult that had not responded to streptomycin

therapy was cured only after additional chloromycetin therapy (32). In other cases (see above and Table 13) a definite activity was however not detectable. The drug is especially recommended by Flamm cited above (73) because it passes easily through the blood-fluid barrier.

On the value of the new preparations erythromycin and magnamycin there are still no clinical reports.

The worst therapeutic results to date were observed in newborns wherein penicillin, or in other instances antibiotics with sulfonamides, proved almost useless. Martinek (316) saw, to be sure, the cure of an infected newborn with the use of penicillin therapy alone. It was recently found that the clinically manifest disease could be curtailed by aureomycin (95) but in a further case of the same authors aureomycin and also sulfonamide remained without effect. Apparently in infections resulting intrauterinely only the strongest medications, that must be given immediately on the merest suspicions (of listeriosis being present), (see Early Therapy under the next section), will have a lifesaving action.

The chronic, protracted listerioses of the CNS are on the contrary easier to control with chemotherapeutic and antibiotic drugs. With simultaneous insulin shock therapy cases diagnosed by the Russian authors as Listeria psychoses showed considerable

improvement after lengthy treatment with penicillin, gramicidin, streptocid, and sulfonamides, so that the patients were able to leave the hospital. The serum titer diminished with the therapy, Lang (312) saw, too, in children ill with listeriosis with mental damage a return to normal of the findings after aureomycin therapy.

It is unconditionally necessary that antibiotic therapy is not discontinued to soon. The concentrations therapeutically attainable act as a rule only bacteriostatically, and the affected organism must itself take over the ultimate extinction of the germs. As already said, the partial intracellular deposition of the bacilli or their capsulation in granulomata may well prevent an effective concentration of the drug at the site of the infection. It is certain, nevertheless that the blood-spinal fluid barrier is easily passed by all the drugs specified.

Too short a duration of therapy results in the appearance of relapses after aureomycin as well as after terramycin therapy (23,308). The case described by Bennett, et al. appeared anew after terramycin. Similar to the not seldom occurring typhus relapses after chloromycetin was thus the reappearance of the activity of the infection not conditioned by increased resistance. The administration of antibiotics should continue for at least 10 days after clinical cure (although at half doses). Unfortunately this indispensable

extension of the antibiotic therapy commonly results in complications such as diarrheas, mycoses, and infections by resistant bacteria.

Besides chemotherapy additional methods must be used according to the nature of the cases (circulatory treatments, lumbar puncture, liver injection therapy, O_2 supplement, etc.) to their fullest extent.

Local infections of the conjunctiva and the throat may be treated locally also in addition to the general therapy which must never be neglected. Silver preparations have proved effective in the form of eye drops in an infection that occurred during labor (5); also a 30% albucid solution, 10% streptocid salve, and 5% xeroform salve was proved good (196). Concerning local antibiotic therapy in the throat one should be held back however because secondary damage by the alteration of the bacteria and fungal flora are not avoidable.

Table 13 gives a review of the results, up to the present, of treatment of listeriosis with sulfonamides and antibiotics:

Table 13. Review of the present chemotherapeutic and/or antibiotic treated human Listeria infections (only bacteriologically proved cases) (concluded October 1954)

Year published	No. of cases	Clinical picture	Age	Therapy	Results	Authors
1937	1	meningitis	adult	Prontosil	cured	Porzecanski & de Baygorria
1939	1	meningitis	child	sulfanilamide	cured	Wagner & Porter
1940	1	meningo-encephalitis	adult	sulfapyridine	cured	Savino
1941	1	meningitis	adult	sulfapyridine	cured	Kapsenberg
1943	1	meningitis	adult	Solu-Dagenan	cured	Harvier, et al.
	1	mononucleosis	adult	sulfapyridine	cured	Webb
1946	1	meningitis	child	sulfadiazine & penicillin	cured	Handelmann
1947	1	meningitis	adult	sulfonamide & penicillin	fatal	Martin, et al.
	1	meningitis	child	penicillin, streptomycin	cured	
	1	meningitis	infant	penicillin & streptomycin	fatal	Sedallian, et al.
	1	typhoid-like picture, pleuritis	adult	penicillin & streptomycin	cured (3)	
1948	1	conjunctivitis	adult	sulfathiazole, sulfadiazine, then irgafen	cured	Beute, et al.
	1	meningitis	infant	penicillin, sulfadiazine	cured (1)	Van Driest
	1	meningitis	infant	1 day sulfadiazine, thereafter penicillin	cured	Slooff
	1	glandular swellings, high fever	adult	sulfadiazine	cured	Felsenfeld
1949	1	meningitis	adult	penicillin, i.m. & i.l.	cured	Myers
1950	1	meningitis	adult	sulfadiazine, penicillin, streptomycin	cured	Berry
	1	meningitis	infant	sulfadiazine, dihydrostreptomycin, penicillin	fatal	Line & Cherry

(table 13 continued)

	1	meningitis	infant	sulfadiazine, streptomycin, penicillin, chloramphenicol, finally aureomycin	cured (2)	Line & Cherry
1951	1	polyserositis, septic granulomatoses	adult	aristamide streptomycin Conteben	fatal	Seeliger, et al.
	1	meningitis	adult	Supronal, penicillin	cured	Seeliger & Leineweber
	1	meningitis	adult	Supronal, streptomycin, penicillin	fatal	Hein
	1	bronchopneumonia, meningitis	adult	penicillin streptomycin	fatal	Ödegaard, et al.
	1	meningitis with relapse	child	sulfadiazine, penicillin, chloromycetin since inactive: terramycin	cured	Bennett, et al.
	2	typical previous history of the mother with listeriosis, detection in the meconium	newborns	penicillin and Supronal	cured	Potel (323)
	1	meningitis	newborn	penicillin, streptomycin, chloromycetin, sulfadiazine, since inactive: terramycin, aureomycin	cured	Line & Appleton
1952	1	meningitis	child	penicillin, streptomycin, sulfadiazine	cured	Tompkins
	1	meningitis	adult	penicillin	fatal	Winkler, et al.
1952/53	3	sepsis, meningitis	newborn	penicillin	fatal	Hahnefeld & Nisolk
	1	sepsis, meningitis	newborn	aureomycin, sulfonamide	fatal	Hahnefeld & Nisolk
	1	sepsis, meningitis	newborn	antibiotics & sulfonamide	fatal	Hahnefeld & Nisolk
	1	sepsis, meningitis	newborn	aureomycin,	cured	Hahnefeld & Nisolk

(table 13 continued)

1953	1	meningitis	adult	streptomycin, chloromycetin	cured	Binder, et al.
	1	meningitis	adult	aureomycin, then relapse	fatal	Gray (308)
	1	meningitis	child	penicillin & Supronal	cured	Flamm, et al.
	1	endocarditis	adult	Supronal	cured (4)	Hoffmann (309) and Walbrück (333)
	1	meningitis	adult	supracillin	cured	Kröger (310)
1954	1	clinically asymptomatic	adult	aureomycin	cured	Myers (319)
	1	meningitis	infant	streptomycin and sulfonamide	cured	Baar (299)
	1	granulomatosis infantiseptica	newborn	penicillin	cured	Martinek (316)
	1	meningitis, pneumonitis	adult	sulfadiazine, sulfisoxazole, penicillin, streptomycin, aureomycin	cured	Finegold, et al.
	1	meningitis	adult	sulfadiazine, penicillin, streptomycin, aureomycin	cured (5)	Finegold, et al.
1953/54	6	meningitis	adults	terramycin, in three cases, 3-72 gm. of sulfanilpyramide or Supronal, sometimes also combined with penicillin.	5 cured 1 fatal	Pohlmann & Boese (301 and 346)

(1) Defective healing with hydrocephalus.
(2) Died after a few months from hydrocephalus.
(3) Cure not traceable to the drug used.
(4) Valvular defects.
(5) With residual ptosis.

6. Activities of public health authorities and prophylaxis.

Today the ever rising frequency of listeriosis in human beings and the fact that the course is not seldom unfavorable leads to speculations as to whether an effective prophylaxis is possible. The haphazardness of our knowledge concerning the distribution and epidemiology unfortunately makes it still very difficult at this time to formulate suitable protective measures because the supposed existence of an active connection with the transmission of listeriosis to humans will first require a careful investigation of the infection in animal herds. Only when the sources of infection are known, will one be able to diminish the prevailing transmission to man or perhaps entirely prevent it.

For this purpose the branches of the public health service must cooperate closely with the veterinary health offices and mutually report on confirmed instances in men and in animals. Just as in tularemia and in brucellosis, lately also in leptospiroses, the permanent registration of proved cases may well be unavoidable. Accordingly, the legal provisions of the decree of the Reich-Ministers of the Interior for the fight against contagious diseases of December 1, 1938 must be broadened.

According to the evidence, professional association with animals brings an increased danger of infection into play (compare Chapter B-6).

This applies also to any case in which animals live in the household with people, particularly to the raising of dogs and cats. Parallels to other zoonoses, for example, brucelloses and toxoplasmosis, are unmistakeable.

Because there is today no active vaccination, we must for the present in prophylaxis be content with general hygienic--sanitary measures. The maintenance of animals should, also for other reasons, be reduced to a minimum. Special precautions are needed if animal keepers, caretakers, and veterinarians must professionally have to deal with sick animals, also slaughterers, workers in flaying houses, and soap factories, in which animal carcases are used, as well as those who follow agricultural occupations belong to the endangered group of persons. Explanations and achievement of increased cleanliness as well as instruction about measures for disinfection that are easily carried out may form, particularly for the rural population, the best prophylaxis.

In case of too great an increase in the number of rats, mice, etc., the outbreak of a series of epizootics must be reckoned with, that may be transmitted to man. As already explained, listeriosis also belongs here. This becomes obvious in the case of the repeated reports of its presence in the East. Systematic rodent control is thereby also to be seen as a weapon in the fight against listeriosis.

The designated role of infected foodstuffs especially of raw milk, perhaps also of meat and game, makes it necessary to broaden control measures in these directions and to sharpen them too (compare Chapter B-6). The use of raw milk and its products is to be avoided in any case if it does not originate from herds that are certified as free from listeriosis. The judging as to whether an animal is latently infected or whether it suffers from an atypical form of listeriosis is, however, not simple. The most certain measure for preventing the transmission of listeriosis by milk is--analogous to bovine tuberculosis and brucellosis--appropriate pasteurization (highheating).

The danger to the fetus and to the newborn as a result of a Listeria-infection during pregnancy that was first published during recent years motivates the necessity for taking a closer look at prophylaxis in this connection. The widespread total ignorance of the fact that the disease is transmitted by direct contact with animals, their excrements, and perhaps also indirectly from (by means of) dust, dirt or filth infection, to man, and in case of pregnancy in which only mild clinical symptoms are manifest by the mother, may lead to abortion and stillbirth, must immediately be done away with by appropriate instruction. The country doctors, but also the midwives and femalesaides as they are many times the only advisors the women of the farms have, here have a great duty to fulfill, but one that will be rich in results. During pregnancy, contact with animals and the

use of raw milk and its products should be avoided as far as possible (157a).

Relapsing inflammations of the pelvis of the kidneys, grippe-like infections with chills, etc., may be the only indications of a listeriosis in pregnancy; and even infections that appear to be banal should never be discounted as unimportant in advising the gravida, since one today knows that among these kinds of diseases a series of infectious diseases may hide that may cause injury to the fetus. For listeriosis these things are more favorably stated than for toxoplasmosis; since one may not only detect the infection bacteriologically or serodiagnostically but can also definitely cure it. In case of basic suspicions of listeriosis during pregnancy medicamentous therapy forms the most efficient means of protection for the fetus.

Thus a patient with purulent Listeria meningitis in the seventh month of her pregnancy was cured by combined supronal--penicillin therapy and was enabled by the weeklong penicillin--supronal treatment to go to term and give birth to a healthy child (242h).

Recently, observations of serodiagnostically confirmed cases of listeriosis during pregnancy at the University Maternity Clinic here have shown that the infection may also be conquered by short-term

aureomycin therapy. The result shows in all cases a drop of the agglutination titer, many times the complement fixation reactions, also become negative, or in a drop in complement fixing titer and in the birth of healthy infants.

Finally we should be reminded that in the field of general prevention of infections in obstetric wards etc., listeriosis must also be considered. The frequent infection of the amniotic fluid as well as the massive discharge of the microorganism in the meconium results in danger to the midwife, the aides, and last but not least to the infant being born healthy. In this sense perhaps certain other groups in the hospital are conceivably in danger, if, at the same time, there is yet no direct evidence for it.

Meanwhile it is noteworthy that diseases in nursing personnel and doctors, sometimes with fatal outcomes, do occur. In a hospital for newborn infants in the USA listeriosis occurred in the infants of two women who were in the same room, and used the same bath (156a). Also the cases of Burn arose in part from a hospital within a short space of time. According to Atkinson five children took sick in 13 days with a meningitis that was suspected as being due to Listeria, 4 of which were fatal (281a).

By immediate early therapy or chemoprophylaxis the endangered newborn may also be protected or cured.

Hereon it was reported (191) that the twin of a newborn that died of listeriosis remained alive with penicillin--streptomycin therapy. Combinations of penicillin with Supronal fulfilled the same purpose in two instances in which listeriosis was proved or was given on the basis of findings in the mother (63). The conquest takes place swiftly by the action of the antibiotics of the tetracycline group, aureomycin, terramycin, achromycin, and tetracyn.

In individual instances one may not always be able to decide whether an infection is present at all. By the looks of things it is however generally possible to decide this, according to the published cases. Accordingly, the medicamentous prophylaxis was carried out for therapy in the earliest stages of the disease or to heal a clinically latent listeriosis.

How haphazard our knowledge still is of the transmission and therefrom of the prevention despite all the publications, may be illustrated by one observation of Patocka (322). The newborn of a female doctor, who denied any illness during pregnancy, and was quite conversant with the problem of listeriosis, died a few days after birth from listeriosis though the mother had a short stay in childbed with no complications. This case shows clearly and precisely how difficult an active prophylaxis may occasionally be.

Conclusions.

Just like other zoonoses, listeriosis is transmissible to mankind. The source of infection is a blind alley, that is, as a rule it occurs in man as a further expansion. The transmission results most often from direct contact with sick animals or their excrements, further from the use of nutrients containing micro-organisms, and from the inhalation of infected dust. In many cases however the source of infection and its pathway remain in the dark. It may be shown that there is increased danger to all persons who work directly or indirectly with animals and handle their raw products. Among the definite hypotheses is included the possibility of an occupational infection.

The listeriosis manifests itself in man as a varying, manysided group of clinical phenomena, that may easily be confused with diseases from other sources. The clinical symptomatology includes grippe-like infections, sore throat with septic glandular involvement, and monocytosis, more or less severe septic generalized infections with predominantly liver involvement, further diseases of the CNS, besides localized mucosal inflammations. Pathologic-anatomically the listeriosis is characterized by granulomatous changes in the affected organs.

All age groups were affected, particularly often however newborns and infants. Clinically inapparent infection in pregnant women lead

diaplacentarily to an intrauterine septic disease of the fetus and with subsequent abortion or stillbirth or to infants barely alive at birth with intra partum infections. The infection can also result from the swallowing of amniotic fluid containing bacteria. The lack of resistance of the tissues, particularly of the digestive tract, facilitates the passage of the bacteria into the fetal body. Involvement of the CNS often presages a fatal outcome. Listeriosis is doubtlessly the source of fetal damage, abortions and stillbirths more frequently than is at present perceived.

Listeriosis in newborns just as the listerioses of the CNS in older persons is charged with a mortality rate of about 70%. Otherwise the course of listeriosis is generally favorable; infections occur that are clinically asymptomatic in man.

Chemotherapy with sulfonamides and lately with the antibiotics of the tetracycline group have considerably diminished the mortality also in the more severe forms. As relapses occur, it is necessary to provide early therapy, a maximum dosage, and a sufficiently long period of treatment.

Prophylactic measures consist, in consideration of the lack of active immunization, of a general improvement of the hygienic and sanitary conditions, particularly of intensified regulation of raw products of animals that are used for human consumption, and of the

instruction of the persons endangered. In case of the suspicion of listeriosis in pregnancy prophylactic medication with antibiotics is indicated.

A diagnosis of listeriosis by clinical means alone is not possible at the present state of our knowledge.

D. Bacteriologic and serologic diagnosis of listeriosis.

With the many-sided symptomatology, the diagnosis of listeriosis by clinical means only is not possible in man or in animals. The symptomatic phenomena under which the disease appears, are too numerous, and in any case other bacteria and noxa could also cause the same symptoms.

Diagnosis during life stands or falls with the direct detection of L. monocytogenes and is definitely established only bacteriologically. Therefore the detection of the microorganism must be attempted in every case, whereupon all the tools of modern bacteriology are involved. The etiologic diagnosis makes possible the assumption of an appropriate therapy and thereby many times is lifesaving.

Cultural methods do not always lead to the goal. In such cases indirect methods of detection may permit valuable conclusions. Bacteriologic and serodiagnostic methods of investigation apply to the diagnosis of listeriosis in suitable fashion and should be carried out in parallel if possible.

Also postmortem an exact diagnosis is possible only in connection with bacteriologic investigations, since tissue changes that are pathological-anatomically identical or at least quite similar may be caused by various noxa and bacteria, and the individuality of the morphologic changes do not unequivocally indicate the individuality of the shaping process.

1. Material for investigation.

Just as the clinical symptomatology is variable, so the material for investigation from which the microorganism may be isolated is also variable. Which material is most suitable in individual instances usually depends upon the clinical picture and the organs afflicted.

In early stages of the septic infection, during its course, and towards the end, also in clinically unclear febrile diseases, mononucleoses, and so on, the blood cultures, repeated if necessary, offer the best possibility for detecting the bacteria. In blood drawn sterilely, best obtained by means of a Liquoid-Venule, the bacteria may be maintained for a month with slow multiplication (314).

Assuming sterile excision or puncture, the Listeria may also be sought during life from glands and by liver puncture (biopsy). Corresponding investigations appear indicated in each case.

Promising, and in individual instances complementing the blood culture, is culture study of the bone marrow (sternal puncture) (319).

Isolation of the bacilli is frequently successful from the cerebrospinal fluid, the ventricular and cisternal fluid, not only in disseminated listeriosis of the CNS, but even in mild meningeal irritation syndromes or a predominantly septic picture.

In cases in which diphtheria is suspected, scrapings from the involved noses, throats, and if necessary conjunctivas should be specially investigated for Listeria.'

This applies similarly in conjunctivitis, urethritis, otitis, etc.

In case of suspicion of listeriosis of newborns the investigation of the umbilical blood, the amniotic fluid, and particularly the meconium allows valuable conclusions (204). If necessary, culture studies must be extended to the placenta and the lochia. Vaginal scrapings and urine investigations ante- and post-partum likewise yield postive results.

Investigation of the mother's milk is also to be recommended.

In the investigation of autopsy material the detection of the microorganism generally succeeds from the liver, spleen, glands, adrenals, heart muscles, brain, medulla and lungs according to the organ predominantly afflicted. Naturally it is possible also with a whole series of other tissues. In aborted fetuses of cows and sheep Listeria may repeatedly be detected in the abomasus, in gravid animals in the uterus, the adnexa, and usually also in the milk glands.

The published examples relate in generally similar fashion to research material in animals and men. With the exception of only a few cases (186e) the detection of the microorganism in animals

took place only post mortem, whereby three basic types were established: a) involvement of the visceral organs; b) involvement of the CNS; and c) combined involvement of the viscera and the CNS (186a). In human infections the detection of the bacteria is conducted with increasing frequency during life.

Also the bacteriologic control of certain animal foodstuffs (unpasteurized cows- and goats-milk, meat, eggs) is indicated.

Bacteriologic investigation of semen is also to be considered for the purpose of avoiding infections during breeding.

As the most propitious time for investigation or for taking the material the early stages of listeriosis are to be so considered when the microorganism is still circulating in the blood stream. The detectable involvement of the reticuloendothelial system and intracellular growth of bacteria in more protracted durations of the illness indicate the beginning of a considerable difficulty in the possibility of cultural detection, and find their parallel in brucellosis. However, cultural studies are also of promise in other stages of the disease particularly during the course of purulent inflammations of the tonsils, meninges, conjunctiva, etc. With the slightest suspicion of the disease in the newborn the detection of the bacteria, should be sought in each case in all available material, and above all else the investigation of the meconium should not be overlooked.

To avoid secondary contamination the usual precautions are to be observed in transferrals. An overgrowth by companion bacteria may be avoided by the quickest possible working up of the materials.

If this is not possible, it is recommended that the material be kept cool, whereby even a certain concentration may be achieved (see p. 113) or quickly frozen and kept in a deep freeze at -25 to -70°C., until the investigation can be conducted.

2. Bacterioscopic investigation.

Only in the fewest cases is a bacteriologic--microscopic diagnosis of the suspicions possible. The performance of gram-staining is presumed. The suspicion of Listeria infection may in case of typical morphology and staining ability of the bacteria only be manifested in the kind of research material on hand that is normally free of bacteria (spinal fluid, fluid obtained by an exploratory puncture (biopsy)) or is at least poor in bacteria (meconium).

There are gram-positive bacilli in the spinal fluid, distributed extra- and intra-cellularly, together with leukocytes and above all mononuclear elements which are suspicious in the highest degree. Figure 20 shows a typical picture.

Not seldom, however, there is confusion with streptococci or pneumococci, resulting from the coccoid appearance of the bacteria and the relatively frequent formation of short chains. In case longer

bacilli are present one has also taken them for diphtheria bacilli. The constant lack of polar bodies and club forms in the case of Listeria is however an important point of differentiation.

Figures 26 and 27. Normal and Listeria-containing meconium in smear preparation (with permission of Potel). Gram stain. Enlarged 800 times.

The meconium of newborns ill with listeriosis often contains the microorganism in great numbers. The abundant presence of gram-positive bacilli is indeed no definite proof of a Listeria infection, but is an important indication that should be made known without further ado to every maternity hospital and every practicing physician.

Worthy of recommendation is the performance of a hanging drop test for the detection of bacteria, and also for the proof of motility in liquid study materials whereby the suspected diagnosis may be confirmed.

In every case however the gram-staining must be performed previous to or in connection with the inspection of the native preparations.

In histologic sections of afflicted organs, one finds the Listeria as individual gram-positive bacilli and also deposited in nests. They may be exhibited also with different methods of staining (see p. 5) and take a silver stain. In connection with typical tissue changes their presence confirms the suspected diagnosis of a Listeria infection.

Bacterioscopic preparations from the nose, throat, urethra, ureter, from the conjunctiva, the urinary sediment, and stools (excluding meconium)

are not of value in differential diagnosis, because in this material the presence, normally, of gram-positive bacilli is already to be reckoned with, that, on the basis of their staining capacity, morphology and arrangements, can not be differentiated definitely from the Listeria (particularly lacto- and acido-bacteria).

3. Methods of Culture.

As long as the bacteria are present abundantly and in pure culture in the research material, their culture offers no special difficulties, because they are relatively undemanding. They thrive well on grape-sugar, blood- or serum-agar and multiply in meat broth and Tarozzi bouillon. Tryptose-agar is also outstandingly suitable for their culture.

Blood cultures made in the usual way (plate molds, inoculation in liquid media, aerobic and anaerobic incubation). The observation time should cover at least 12 days. Interim readings after seeding on blood- and tryptose-agar made on the 2nd, 4th, 7th, and 12th day.

Other liquid material for investigation is centrifuged for 15 minutes at 3000 revolutions in order to concentrate the bacteria. The sediment is, after use of part of it for making object-glass smears, spread on the surface of blood-agar or one of the named substrates, and partly for inoculation into liquid media. A small quantity should be kept in the refrigerator for further study. All

inoculated media were incubated aerobically, occasionally in the presence of a 5-10 percent CO_2 atmosphere for a total of 3 days at 36-37°C. and observed every 24 hours. From the liquid media which show a cloudiness subcultures may be made on solid media.

Meconium is worked up directly without any other previous preparation. Listeria do not grow on the special media for culture of Enterobacteriaceae.

Many report difficulties in the culture from tissues, such as bits of organs from biopsy specimens, resected material, etc. In order to avoid contamination the specimen must be obtained, according to rules of the trade, under sterile conditions (with the use of sterile precautions). The material for investigation is taken from the interior after singeing the outer surface. The bacilli do not always grow on solid media. Preliminary cultures in liver and in tryptose-bouillon give better results; thioglycollate-dextrose-bouillon (298) also renders outstanding service.

Brain pulp is inoculated directly on the surface of the solid media and into liquid media.

The microorganisms do not infiltrate the tissues evenly and are partly deposited intracellularly also. The extraction of material from bits of organs with the platinum wire loop is thereby undertaken with considerable chance, that could be largely avoided if the tissue material

is basically homogenized before the inoculation. This is done by pulverizing or mechanically by means of tissue grinders.

The more material is investigated, the greater usually is the yield. In spite of all precautions, however, the culture yet is not always successful. This may partly be traced back to the presence of inhibitive substances in the research material itself, but has its cause more often, however, in that in the beginning too few bacteria were present (blood, brain, medulla, etc.).

Therefore the individual authors employ the biologic investigation, whereby suspected material is inoculated into susceptible animals and the detection of the bacteria is then carried out from the organs.

Thus Bilibin was able to obtain Listeria in pure culture from the organs of guinea pigs that had been infected with the venous blood of his patient, although the direct cultures remained without results.

Naturally the research animals themselves must be definitely free from the disease or the microorganisms.

Olson and coworkers have on the contrary determined in a distinguished series of studies, that the detection of the microorganism by inoculation of mice was less successful than preliminary culture in liquid media and seeding on tryptose-agar (86d).

In 114 preparations, Listeria were cultured 25 times on tryptose bouillon, but only nine times from artificially infected mice. In no

case was mouse inoculation successful when the direct culture did not grow. Perhaps the poorer yield of the mouse passages results from natural resistance of the research animals, which enable them successfully to overcome infections in small doses.

It may be concluded that direct culture methods are the means of choice, assuming suitable processing and use of the best media.

Meanwhile the difficulties of culture with small numbers of bacteria are not fully excluded. Gray and others report on this that cultures grow only in a third of the histopathologically proved cases. Through the use of a simple method of enriching them, the culture yields may increase considerably. Gray, Stafseth, Thorp, Sholl, and Riley hereby make use of the ability of Listeria, that was confirmed by numerous investigations, to grow at low temperatures (87a).

A part of the research materials were immediately processed in the manner mentioned, the remainder was kept in a refrigerator at 2-5°C. for a few weeks to 4 months. At intervals of 2 weeks a small quantity was again renewed on blood- or tryptose-agar or inoculated into liquid media.

The results were manifested by a steady increase in the number of growing Listeria colonies. In a control experiment the American authors found in material that yielded results that were in the beginning negative already after 10 days 220, after a further 8 days

exactly 1400 Listeria colonies.

The successful increase in cultural yields in this manner is considerable; it amounts to 30% (314) and sometimes even to 95%!

Cultural investigations of research material that normally contains bacteria such as stools, milk, throat swabs, lochia, etc., are many times negative, because the concomitant flora overgrows the slower growing Listeria. Possibly bacterial antagonisms also play a role. In many cases here the culture on blood or tryptose agar in a refrigerator at 2-4°C. is successful.

Generally under these conditions saprophytes, e.g., the Proteus bacteria and the Enterococci, also multiply.

The selective media for Listeria has not been found to date. The use of sodium azide ($NaCON_3$) in concentrations of 0.03-0.2% yielded no profit (87b). The same authors found however that potassium tellurite even at concentrations of 0.1% still permitted growth of L. monocytogenes strains. In the presence of 0.05% of potassium tellurite the bacteria grow unhindered because of the simultaneous inhibition of gram-negative bacilli. Micro- and strepto-cocci generally multiply also at this concentration.

With the use of a meat broth bouillon with the addition of 0.05% potassium tellurite Gray and coworkers achieved good results.

After 24 hours of preliminary culture inoculated tryptose-

agar plates showed many times a growth of Listeria in pure culture, even if artificially infected material such as straw, stools, nasal scrapings, etc., were investigated. It was generally assumed that not too many cocci were present in the beginning.

This important finding was repeatedly confirmed. With the tellurite--containing medium that was quickly adopted by us we found that the Clauberg--agar in the modification of Herrmann was good for the selective cultivation of Listeria. The authors could, in this manner, cultivate the bacteria from highly impure necropsy material even after 5 months. Schabinski was successful in cultivating a Listeria strain from throat swabs by using the tellurite plates according to Schroer.

Lately however it has been demonstrated that a few strains of Listeria do not tolerate 0.05% potassium tellurite (186d).

Crystal violet is not suitable for the selective culture of L. monocytogenes even at low concentrations.

According to Shimizu and others, on the contrary, media containing guanofuracin (5-nitro-2-furfurylidine-aminoguanidine-hydrochloride) have proved good. The concentration amounts to 1:12,000 in dextrose bouillon as an enriched medium and 1:10,000 in 5% sheep blood agar as a selective medium. The detection of Listeria is made

still easier by the addition of 0.01% potassium tellurite. This specific solid medium will completely inhibit gram-negative bacilli, sporeformers (fungi?) hemolytic streptococci and staphylococci, but will not prevent the growth of Streptococcus viridans and enterococci. On the guanofuracin-tellurite plates the Listeria colonies are black and have a gray-green edge, while strepto- and micro-coccus colonies are seen as pink-gray or gray-blue.

Storage of the research material in 50% glycerin at 4°C. (29) for over 2 months made no appreciable difference.

Compared to culture results with fresh material the yield was cut in about half (from 93 to 47), the proportion of undesireable concomitant bacteria only 17% (142 to 119). Meanwhile the culture was positive in some cases only after combined glycerine-refrigerated storage (186d).

In short, it has been shown that by a combination of different methods of selective culture of Listeria is possible only to a limited extent.

Perhaps for the successful achievement of further improvement, the addition of subthreshold quantities of penicillin or the use of higher concentrations of sodium chloride or preliminary culture in alkaline media are required. --In connection with this the observations of Flamm (306) are of interest, that even 2 1/2% sodium chloride in

dextrose-agar resulted in total inhibition of the growth of Listeria, in contrast to the good growth of these bacteria in fluid media with 10% NaCl.

However it must be considered in any case that other organisms, particularly the Enterococci, behave culturally similarly to the Listeria.

4. Comprehensive diagnosis and differential diagnosis.

In view of the systematic position of the Listeria in the bacterial kingdom that was for a long time indefinite, and their manifold similarities to other kinds of bacteria with regard to morphologic as well as cultural aspects, it is understandable, that they--even by experienced researchers--are commonly overlooked or are otherwise classified. It has been shown in continuous repetition in various countries that the first reports and writings are soon followed by other reports, as soon as the boundaries (or limits) of the Listeria can be defined.

The comprehension and detection of suspected strains assumes the exact knowledge of their properties, but particularly their cultural characteristics. The recognition of questionable strains is assured only by surface culture on semisolid media, occasionally after preliminary culture in liquid media. Blood-agar plates have proved the best for this purpose.

The growth is at first sparse and after 24 hours is hardly visible. With respect to the colony form, that is elucidated on pages 9 ff. The characteristics of the Listeria colonies can easily lead to their confusion with superficially similarly growing colonies of other bacteria, for example with streptococci, diphtheria bacteria, other Corynebacteria, etc. The important characteristic of beta hemolysis is not always well defined. Therefore it is no wonder that enterococci, lactic acid bacteria, erysipelas microorganisms, etc., are confused with Listeria.

The diagnosis of colonies on blood-free media, such as tryptose-agar, is more difficult, because then the important characteristic of beta hemolysis is absent. Gray and coworkers report good results here with the use of the plate microscope.

If white light is allowed to shine upon the Listeria colonies from beneath at an angle of M 45°, they will shine a bright green when the plate microscope is used. This behavior is thus characteristic, that they may be identified even in extremely contaminated cultures.

If the medium contains potassium tellurite, the colonies appear black. In contrast to the cocci colonies that are sometimes black, black-yellowish, and gray, the Listeria colonies appear, with this kind of lighting to have the typical greenish coloring in the border zone.

Inasmuch as the bacteria are not already grown in pure culture, it is feasible to transfer suspicious colonies for purification onto fresh blood- or tryptose-agar first. The characteristic colony forms are so widely distributed after 24-48 hours incubation at 37°C. that they may be detected with the naked eye, but better with the plate microscope. Now one makes the smear preparations and tests the staining capacity according to the method of Gram as well as the morphologic behavior.

The detection of typical encapsulated lancet-shaped cocci, typical chain cocci, pleomorphic Corynebacteria, with polar bodies, exclude bacteria of the Listeria group with certainty.

Definitely significant is the determination of motility.

We make for this purpose either a hanging drop of a young bouillon culture incubated at room temperature or observe the growth at 22 and 37°C. in 0.2-0.4 percent nutrient agar stab (so-called motility agar).

Non-sporogenous, gram-positive, beta-hemolyzing bacilli, that are unequivocally motile, and present the characteristic stratified zone of maximum growth about 3-6 mm. beneath the surface of the agar column belong almost without exception to the Genus Listeria.

To confirm the diagnosis the control by means of a color series, of the most important biochemical characteristics (see p. 12) is necessary. Also worthy of recommendation is the agglutination test in a polyvalent Listeria antiserum. The possibilities of error here

(see p. 21) are, however, particularly large, so that caution is needed. Indispensable for all doubtful strains is the determination of pathogenicity for animals (see p. 29) and the genesis of monocytosis in research animals with carefully graduated quantities of bacteria.

The author and Linzenmeier (2421) have formulated the following criteria that are reliable aids in the differentiation of the morphologically or culturally similar organisms:

a) Erysipelothrix-group: Morphologically similar--on blood agar no beta-hemolysis--nonmotile--no esculin splitting--slow decomposition of dextrose with little acid formation--characteristic growth in gelatin stabs at 22°C.--no agglutination in Listeria sera.

b) Lactobacilli: Aerobically on blood agar only sparse growth; no beta-hemolysis--catalase negative--nonmotile--confusion possible, because of similar morphology and similar odor of sour milk--serologically sometimes spontaneously agglutinable, sometimes not agglutinable in Listeria sera--not pathogenic for animals.

c) Streptococcal group: Morphologically generally easily differentiable--more difficult in case of atypical strains with rodlike forms carefulobservation of the hemolysis--Listeria show no alpha or gamma hemolysis--beta-hemolyzing streptococci in colony form are only superficially similar to the Listeria.

Beware: Confusion of Enterococci with Listeria! In case of

enterococci, there is generally destruction of mannitol--serologically there are overlapping reactions--Enterococci and nonmotile. Otherwise great similarity!

Differentiation of motile strains of streptococci--morphologically, color series--if necessary, animal investigation.

d) Pneumococci: With attention to typical characteristics confusion culturally hardly possible. Bacterioscopically (for example in spinal fluid) the diagnosis of pneumococci is made only if definite lancet forms and capsules are detectable. If possible checking up by Neufeld's swelling test.

e) Corynebacteria: Morphologically generally definitely distinct--few little known kinds are however somewhat similar--practically always nonmotile--special caution is required in the case of all beta hemolytic growing strains--possibility of differentiation in color series see Table 14.

Beware: Confusion with motile C. poinsettiae and related kinds; these liquefy gelatin and form orange colored or reddish pigment--not pathogenic for animals--serologically differentiable from L. monocytogenes.

The differentiation of strictly anaerobically growing Corynebacteria species is performed by their cultural characteristics.

Finally it appears that there is a series of similar kinds of bacteria that may not be identified with relations of the abovenamed

groups, that are also however differentiable from L. monocytogenes. The determination of the species or type classification of these kind of bacteria is difficult.

A culture that belongs here was for example described by Schier as a non-pathogenic Listeria. Stanley also found Listeria-like organisms in throat swabs, and Seeliger did likewise in urine and punctates from pleuras. Murray sent the author a strain that showed the following properties:

On blood agar round, small colonies with significant hemolysis (turning green on sheep blood agar--author). Microscopically short gram-positive coccoidbacilli, non-motile. Acid formation from dextrose, maltose, galactose, and salicin, irregularly from lactose, mannitol (!) and saccharose. Decomposition of esculin, catalase weakly positive. No H_2S-formation, urea hydrolysis, nitrite formation or gelatin liquefaction. Non-pathogenic for research animals, not monocytogenic, negative Anton-test, serologically not related to L. monocytogenes.

Murray believed that these bacteria belonged to the Listeria group and supposed that here there was a special type of Listeria distinct from L. monocytogenes. Possibly this is identical to the bacteria described by Hülphers (317). We usually observed with the abovenamed cultures definite chain formation and never motility, while Murray observed indications of motility. Therefore we must leave the question of the classification open at present.

Table 14. Differentiation of L. monocytogenes from aerobic Corynebacteria and Erysipelothrix-species.

Species of Bacteria	Beta-hemolysis	Motility	Decomposition of					Exotoxin
			Urea	Esculin	Nitrate	Dextrose	Salicin	
L. monocytogenes.....	+	+	-	+	-	+	+	-
E. rhusiopathiae.....	-	-	-	-	-	+	-	-
C. diphtheriae.....	+/-	-	-	-	+	+	-	+
C. belfanti (27).....	+	-	-	-	-	+	-	-
C. ulcerans.....	+	-	+	-	-	+	?	+
C. hoffmanni.....	-	-	+	-	-	-	-	-
C. xerose.....	-	-	-	-	-	+	-	-
C. pyogenes.....	+	-	-	-	-	+[1]	-	+
C. ovis (Preiss-Nocard). (C. pseudotuberculosis)	+	-	+	-	-	+	-	-
C. kutscheri.....	-	-	-	+	-	+	-	-
C. equi.....	-	-	+	-	+	-	-	-
C. renale.....	-	-	+	-	+/-	+	-	-
C. poinsettiae.....	-	+	-	+	-	+	+	-

1) serophil.

Still a few further remarks about the antigen analysis (for particulars see page 14 ff.).

The determination of all O- and H-antigen factors requires a large number of sera and should be carried out only by experts. Object glass agglutination of living bacteria must be performed with caution, since spontaneous agglutinations are frequent. The same applies to heat-treated antigen. Artificial agglutination of heterologous bacteria takes place in Listeria serums. Serologic type determinations are useful only after or in connection with concomitant cultural and biochemical investigations. The accumulation of suspicious strains at a central establishment is important on epidemiologic and rational grounds and is to be urged.

5. Serodiagnosis.

Generalities: In view of the hindrances which stand in the way of the cultural investigation of the microorganism of listeriosis, and are not very avoidable even with the best research methods, serodiagnostic procedures possess a more than theoretically-scientific interest. Their significance extends to the retrospective diagnosis of previously survived infections but also to the detection of acute or chronic illnesses that are diagnosable bacteriologically not at all or only with difficulty and from those to the epidemiologically important determination of the distribution of the disease in general.

The basis for serodiagnosis is the fact that the infected body during the course of the immunobiologic exposure forms antibodies to the microorganisms that, by the use of certain methods of research, are detectable qualitatively and quantitatively in vitro.

It should not remain unmentioned that the sources of error in indirect methods of detection are incomparably greater than in the case of bacteriologic methods. Therefore these kind of studies give promising results only when they are carried out in a uniform manner and therefore with comparable techniques at the sources, that with tested methods and sources of error determined, have at their disposal extensive, comparable material.

The evaluation of serologic findings is to be carried out with great caution and reserve, and one must, in addition to epidemiologic characteristics, consider the clinical picture. Indeed, in many instances no definite conclusion is possible above all, if only a single test is performed. Only the results of tests repeated at intervals and controlled yield useful findings and permit diagnostic inferences.

The serodiagnosis of listeriosis stands only at the beginning. This stems chiefly from the fact that the earlier researches were generally carried out with total absence of uniformity of the test conditions and the differences of the control-materials many times

contradicted the findings and often yielded results that could not be compared.

Seroanalytic investigation of Listeria antibodies must take into consideration the differences in antigen structure of the Listeria and correspondingly must be conducted with antigens of the individual types, comparable, according to which method is used.

The preparation of unobjectionable antigens is not simple. Before their use they must be tested in agglutinizing and in negative sera. To each daily mixture belongs positive and negative controls. The test conditions must be uniform.

Sufficient constancy is assured among other things by the manufacture of large batches of antigen, which will be used over a long period of time, and are capable of being held for many months because of correct manufacture and keeping in refrigerators. The initially established dilutions for use should be strictly adhered to. Duration of incubation and techniques for dissolution should not be varied.

In analogy to the serodiagnosis of the other bacterial infectious diseases it is recommended for use at least for the agglutination test, the international uniformly determined standard strain, and also to carry it out with antigens manufactured by standard processes. Only thus can comparable results be achieved.

a) Widal-reaction. Here it is a question as to a sero-analytic investigation of agglutinizing antibodies against the O-and H-antigens of the different types of Listeria.

This test has been investigated most frequently to date. Unfortunately earlier investigators have not always taken the antigenic differences into full consideration. Sometimes in a series of studies only antigen of a single strain were used, which was naturally appropriate in individual instances if it is a question of a previously isolated microorganism. --Meanwhile it is certain that with one antigen agglutinins against heterologous types will not be affected or only partially so. Living or dead total antigen is often used, so that it is many times not possible to say whether the antibodies that were found reacted against the O- or the H-antigen.

The technique for the preparation of indubitable O-or H-antigen was described on page 17 ff. Correspondingly with the diagnostic antigen formulae one must use O-antigen of the types 1,2,3, and 4 and the H-antigen of types 1 (sometimes also 2) and 4. Greater value is to be laid to the pH of the antigen precipitates, which must react neutral or weakly alkaline (pH 7.0-7.4) because the antigens are spontaneously agglutinable in acid media.

Research techniques and techniques of dissolution are explained on page 18. For routine diagnosis the tube test is quite well suited. For the lowest serum dilution we use a serum concentration of 1:40

after the addition of the antigens. Orienting studies have shown that the Widal test for listeriosis is capable of being performed on object glasses also with greater savings of time and materials.

One retains hereby technically for the foregoing, that they are performed with the use of correspondingly concentrated antigens as in the case of the serologic diagnosis of brucellosis. These methods were used for the diagnosis of Listeria antibodies by Graham and coworkers in connection with a similar study in the case of erysipelas (230).

To save time one may also use the centrifuging method of Gaehtgens, which has proved particularly good for the detection of antibodies against Salmonella, Escherichia pneumococci, and other antigens (37a). The titer here is generally higher, but cannot be however, due to the frequency of Listeria agglutinins, looked on as significant.

The serum to be investigated should be as fresh as possible. In general it is not necessary to inactivate serum before the Widal test. In a few studies, however, occasionally a definite difference was shown in cases of the agglutination titers of active and inactive sera, so that it may be important to conduct them simultaneously always. To answer the question as to whether active or inactive sera give more specific results in the agglutination test, further research is necessary.

A series of human and veterinary medical reports yield conclusions on the agglutinizing Listeria antibodies in serum of healthy and sick persons and animals without it being generally possible to date to develop uniform guiding lines for the evaluation of these kind of findings.

Thus Nyfeldt found in his patients titers up to 1:512, and Beute and coworkers saw a titer rise up to 1:1600 during a period of 14 days. The author observed in bacteriologically confirmed cases of listeriosis or clinically suspicious cases repeatedly O- and H-titers between 1:320 and 1:2500. Similar observations are found in many other publications (23,40,41,99,139,151,156a,196,204,242h,268, 274,281,326,337,344,346,349,349a, and others).

But on the contrary there are verified cases of listeriosis in which the detection of agglutinizing antibodies was not successful in sufficient titers (23,40,70,99,204,281a, and others).

Debatable and unclear is also the significance of Listeria-agglutinizing antibodies in sera of healthy people or of patients without any history that can be connected with the presumption of a listeriosis.

Webb saw in control sera titers up to 1:125 once even to 1:500. Seastone reported on average titer of 1:40; and Julianelle observed in 46% of the tests (including patients with mononucleosis) Listeria

titers between 1:20 and 1:160. French authors (99) found in four of 12 sera titers between 1:100 and 1:300. This corresponds approximately with the results of our own research, (242j) on almost 700 sera of which about a third manifested O- and H-agglutinins against one or more of the Listeria types, mostly generally in the dilution of 1;80, seldom on the contrary higher. Deviating from this was the control in the case of 120 normal sera, (from city folks) in which positive agglutination was found only once (188b).

The situation is also similar in the animal kingdom.

In individual cases, for example in cows sick with listeriosis, high titers of 1:5000 to 1:50,000 were detected, in others, in which the disease was similarly determined bacteriologically, the agglutinin formation remained among the missing (86b;298). The formation of specific antibodies was observed also in artificially infected pigs. --During an epizootic among sheep Hirato and coworkers found in the case of 18 of 71 sheep investigated 54 days after the last occurrence of the disease in the afflicted herd, titers of 1:800 and over. Yearling sheep had titers of under 1:200. Older animals mostly were over 1:200. In most of the seropositive animals the titer was hardly changed 7 weeks later.

In serum of healthy animals without ascertainable exposure Listeria agglutinins were found very often, for example in horses, cows, rabbits, guinea pigs, sometimes even in high serum dilutions.

Outstanding results were supplied by the remarkable series of researches in Halle, whereby (61,204,323) for the first time animals in Germany were investigated too. Positive agglutination results were found by Potel and Ehrenhard in the following frequency:

Horses	25 times (61%)
Cattle	186 times (34.5%)
Pigs	18 times (28%)
Dogs	3 times
Sheep	1 time.

The titer amounted to, with the use of a living total antigen of type 1, 1:200 and more. 27% of the horse serum yielded a strong agglutination at dilutions of 1:1600 and over. --Brucellosis titers and leptospirosis titers had no influence on the Listeria titers.

In view of the abundance of Listeria titers in men and animals that are positive the question raised as to their significance and specificity cannot be definitely answered now. To date there is no conclusive proof that Listeria-infections are that common. In any case Graham, Hester, and Levine 1941) concluded: "There is no agglutination test, complement fixation test, or precipitin test of proved value in the diagnosis of the natural disease".

What can the surprising frequency of Listeria agglutinations be attributed to them?

They could be based on the antigen itself, especially as many strains are spontaneously agglutinable or at least show this tendency under certain cultural conditions. But also after the exclusion of these sources of error there are frequent enough observations of unequivocal agglutinations, and indeed in practically the same ways, with antigens of different strains of the same type in case of negative findings in other sera. The hypothesis of a non-specific sero-agglutinability or lability is hereby unlikely.

The frequency of positive findings is also not explicable by a too great sensitivity of the method, since a considerable portion of the sera daily investigated give negative results under like conditions.

Thus the hypothesis that remains most probable is that the cause lies in the sera themselves, because the agglutinins may be entirely removed by saturation with Listeria antigen. They are not present in the same quantities in the various age groups. Just as in young animals Listeria agglutinins are not detectable at all or are present only in small quantities, the sera of newborns and infants usually react negatively. Only from the end of the first year of life onwards are positive findings more frequent.

Thereby arises the question as to what extent specific Listeria antibodies are involved. Also here a definite answer is not always possible.

For example, it is unclear whether during the course of other diseases heteroagglutinins against Listeria-antigens appear, corresponding to the Proteus OX_{19} agglutination in typhus (8) or the serologic interrelationship between Bang's disease and tularemia titers and vice versa.

Furthermore, the possibility must be considered, that the invasion of the body by bacteria that are harmless to it, for example intestinal bacteria, may lead to the formation of antibodies that can also agglutinate Listeria-antigen. For this however, extensive studies of the antigenic relationships between Listeria and other kinds of bacteria will be necessary. A number of reports show, to be sure, that certain strains of Enterococci of the Lancefield Group D have partial antigens in common with Listeria (2421).

This was confirmed by reciprocal agglutination--and absorption--tests in immune sera. It was also found that human sera with Listeria titers regularly agglutinated the corresponding Enterococcus strain. But after absorption with enterococcal antigen there remained now always detectable the specific Listeria-agglutinin.

Through the researches of Nyman it is known that a high percentage of human sera contain agglutinins against one or more of the enterococcic types, of which to date 17 (180) or 25 (90) are known. It is likewise indefinite here as to just where the limits of specificity lie.

It is quite plausible to think that still further antigenic properties exist, which cause heteroagglutinations of different kinds of bacteria after formation of humoral antibodies. Also there should be included here the cross-reactions between Listeria and many Staphylococcus aureus strains (56a, 242j) the significance of which for the serodiagnosis of listeriosis is still to be explained.

The limitations of serodiagnosis are on the whole shown therewith. The specificity is only certain then, if the microorganism itself can be found and antibodies against the diverse antigens can be measured after the manner of a titer curve. Without direct detection there remains a factor of uncertainty, that is more or less considerable.

These circumstances have their parallel in, for example, the Paul-Bunnell test, by which the differential diagnosis between hemagglutinations caused by viruses and those caused by other forms is possible only through tedious saturation studies. It is likewise the case with the Listeria-titers of which the specificity is revealed only after saturation with enterococcal antigen and vice versa.

It is therefore understandable that numerous authors take a position in at least skeptical opposition to the investigation of a serodiagnostic test for listeriosis by means of an agglutination reaction.

Meanwhile extensive research has demonstrated that during the

course of Listeria infections high O and H titers not seldom appear, so that these kinds of titers, whereby strong agglutinations of O- and H-antigens are to be found at serum dilutions of 1:320 and over, also acquire a certain power of proof in case of failure to detect the bacteria. Isolated O-titers may be considered in the same sense, according as it is asserted by the Russian authors, that Listeria-agglutinins remain detectable a year after the disease. Titers of about 1:160 form the limiting value (borderline value).

Things are the simplest whenever, during the course of a disease suspected as being listeriosis, regular O- and H-titer curves appear, with rises in the titers of more than two steps.

The presence of heteroagglutinins, etc. does not mean, however that lower Listeria titers are of no importance. As already said, there results for reasons not known, in bacteriologically proved cases of listeriosis, occasionally only a slight formation of agglutinin or none whatever. Stanley saw during the course of listeriosis this kind of low titer rises, up to 1:32 or occasionally up to 1:128. In our own observations "normal titers" up to 1:80 are obviously more frequent in country folks than in city folks (similar to the titer values in the Sabin-Feldman test in the diagnosis of toxoplasmosis). In the same sense are the findings of Lang to be considered, who has called our attention to findings concerning the presence of the same

kind of higher titers (over 1:320) in mother and child. A significant difference in the distribution of agglutination titers by sex was not revealed in the testing of 449 subjects (151). Moreover the findings of qualitative agglutinin analysis corresponded closely to the regionally determined prevalence of the Listeria types.

In West Germany for example the Listeria type I is most frequently found, (everywhere, with the exception of the strains isolated in Wuppertal and Hamburg). Analogous to that one finds with striking frequency type 1-0 agglutinin in the sera tested. The H-agglutinin reacts on the contrary almost constantly against the H-antigen of type 1 and 4 which because of the great similarity in H-antigen structure of these types is not surprising, and at the same time speaks for the specificity of the titer.

In any case the low Listeria titers in man and animal would also be of a not to be underestimated epidemiologic and epizootic significance, if they should be actually specific.

The serologic differential diagnosis between listeriosis and other infectious diseases must reckon with the frequency of normal titers. In tularemia cases isolated Listeria titers are observed, indeed about in the same percentage as in control persons. Experimentally an antigenic relationship between Listeria and P. tularensis may be definitely excluded (242j). Patients with typhus and paratyphoid show no rise in Listeria titer. Not seldom one finds in the serum of

patients suspected of having listeriosis a positive Sabin-Feldman test and vice versa. This results, as far as we know today, not from an associative influence of the seroreactions by the Listeria or toxoplasma antigens, especially if sera with high titers are also found, in which only one test, that is, the Listeria-Widal or the Sabin-Feldman test, is positive. In case of the widespread, similar, epidemiology and epizootiology one must in addition to these reckon with double infections.

In view of the present obscurities and difficulties the question remains as to whether the Widal test can be generally recommended in listeriosis. Uncritical evaluation of the serologic findings must lead to clinically and epidemiologically false conclusions. By critical evaluation, on the contrary, the serologic findings can give the clinician valuable hints. In view of the importance an immediate recognition of the clinically hidden Listeria infection during pregnancy, the conduction of the investigation should by no means be overlooked, however difficult the explanation of positive results may also be.

This similarly applies to other course forms, in which the bacteriologic detection is no longer successful. High Listeria titers in a clinically obscure septic-encephalitic disease directs the attention to listeriosis, and the indicated antibiotic therapy

leads not only to clinical healing, but also to a steady diminution of the Listeria titer (151). These examples can be multiplied in which repeatedly the life of the newborns could have been saved, if the Listeria infection had been discovered and cured early in the gravidae.

In the early stages of listeriosis on the contrary the detection of Listeria antibodies is hardly to be expected, since agglutinins are detectable at the earliest 7 to 10 days after infection or the outbreak of the disease.

It appears that the listeriosis-Widal test is not specific and in doubtful cases, must be supplemented by saturation studies (for example with enterococcal antigens). The somewhat difficult interpretation of the results gave rise to a large amount of criticism and reservations. Only with these assumptions does the Widal test appear to us to be of value in the current diagnosis. We believe it is valuable, and pertinent nevertheless, if it is supplemented by one of the subsequently described methods.

b) Complement fixation test. The notorious difficulties in the determination of the significance of Listeria agglutination titers gave impetus to the use of the complement fixation test, after good results were accumulated with this technique in the case of other zoonoses, (especially leptospirosis and toxoplasmosis).

Graham and his coworkers were of course the first to try to

find complement-fixing Listeria antibodies, but, as already stated, without results. Later Stanley in his investigations of Listeria lipoids set forth anew the complement fixation test, that because of the lack of serologic activity of the monocyte-producing-agent (see p. 27) also gave negative results. Kolmer found, that immune sera against L. monocytogenes did not react with the antigens used in the Wassermann test.

Independently of one another Oezgön and Seeliger then reported on a complement fixation reaction in the presence of Listeria-antigens and came to practically corresponding results following investigations with rabbit immune sera and human sera, which showed, that complement fixing antibodies are detectable in immune sera even at high dilutions but are absent in normal sera. The author found concerning this furthermore, that the complement fixation test in the presence of the antigens of the microorganism reacted positively also in the serums of patients, and concluded from this that the technique was of diagnostic value. Later investigations on more than 1000 normal and patient sera, among which were included bacteriologically proved cases in all the age groups, confirmed the correctness of this conclusion in case of listeriosis in pregnancy, listeriosis of the CNS, and other course forms. (242j,315). Patocka and coworkers came to almost the same results with a complement fixation test for listeriosis, for the early confirmation of clinically

hidden Listera infections during pregnancy.

The lack of host-specificity of the Listeria and their serologic differences, in antigen formation requires the use of different antigens. Some authors (188b, 191, 323) used, by way of example, only antigens of serotype I, the author in the beginning used those of type 1 and 4. On the further extension of the project that began in 1951 it was shown that antigen of type 3 is also not unnecessary. Besides, lately, control studies with antigens of the Listeria-agglutinable enterococci types have become necessary.

As there is no marketable extract and antigen, these must be made and standardized by the investigator himself.

The number of antigens necessary as well as the time-consuming and tedious, preliminary research make the test unsuitable for small laboratories. It should, therefore be carried out--similarly to leptospirosis tests--only in suitably equipped special laboratories.

The antigen preparation follows extensively the procedure for the manufacture of Listeria-O-antigen, because the flagella material obviously has no special significance in the complement fixation test. Examples may be found on p. 17. Antigens treated with ultrasonics are more active than suspensions that have not been subjected to ultrasonic vibrations and are easier to manipulate as a result of

their suspension stability and the absence of spontaneous formation of clumps. Besides, more antigen is liberated during the ultrasonic procedure into the solvent than during the heating process alone. In principle it is the same, whether the total antigen is used for the complement fixation test in the form of homogenized suspensions or the clear, supernatant fluid remaining after centrifuging.

The selection of the test strain necessitates great care, because the antigen content is subject to certain variations, in connection with the phase of the culture. It is besides strongly influenced by the conditions of the culture.

All strains must be found in their culturally smooth forms, and are from time to time to be tested for their virulence. Because even among the same serotypes, quantitative differences in antigens are not rareties, we are thereby required to use only the standard test strain used in the Widal test. We thereby produce for ourselves an antigen mixture of homologous type strains, into which we at times mix five to ten serologically analyzed cultures of the same type. Thereby chance variations in antigen stability may be largely avoided, since the use of this antigen considerably enhances the constancy of the results, and the charges made at different times show more uniform results in the immune sera.

In place of total antigen one may use for the detection of

complement fixing antibodies in rabbit sera also chemical extracts of the bacteria.

Quite suitable for this purpose of preparing the extracts is the method of extraction with trichloracetic acid in the cold according to Boivin and Mesrobeanu. Finally it is neutralized with NaOH. The antigen, which demonstrates a polysaccharide composition, may be purified by precipitation with 4 volumes of ethyl or methyl alcohol and is distinguished in tests with rabbit immune sera by a slight rise in specificity in case of the diminishing titer values.

Naturally one must, in the presence of definite results in rabbit immune sera carry over into human sera without further ado.

Thus it is for example known, that purified polysaccharide fixes complement in the presence of specific immune sera of rabbits, while under the same conditions the test results are negative in human immune sera. These facts were ascertained in pneumococcal infections and also apply to fungus infections and it has not been excluded that similar conditions apply to listeriosis also.

Therefore it appears just now less pertinent to use polysaccharide extracts in the diagnosis of human listeriosis. Its manufacture besides requires much work and the yield of active substance is often quite small.

All antigens and extracts retain their serologic activity after the addition of a suitable disinfectant (for example, phenol in an end concentration of 0.5%) in case of storage in the cold for many months.

In case of correct preparation they are not hemolytic. For control the concentrated antigen is incubated for 12 hours together with amboceptor--erythrocyte mixture.

The standardization of the antigen is conducted against homo- and heterologous rabbit immune sera in such a way that the same antigen concentration is determined for the sample dilution that yields with the same type of sera the highest titer consistently in at least three tests performed on different days, and reacts in the heterologous sera with the least possible overlapping. The sample dilution is a fixed amount and may not be arbitrarily varied, even if the suspension as a result of increasing autolysis later does not appear as cloudy as in the beginning.

The preliminary research does not, according to principle, deviate from the technique for the complement fixation test in the presence of other bacterial antigens. After determination of the complement titers against the hemolytic system the complement consumption is tested in the presence of the standard dilution of the different antigens. Such a procedure for an antigen is illustrated in Table 16. All reagents are stored until they are used in a refrigerator or in ice water. As a diluent a veronal buffered NaCl solution is commonly used. The fixation time is 1 hour at 37°C. in a water bath. It is read off 15 minutes after the addition of the erythrocyte--amboceptor mixture and incubation in a waterbath at 37°C.

Table 16. Research protocol for the determination of the consumption of complement by antigens from L. monocytogenes (Example: type I)

Antigen standard dilution	Amount	Veronal buffer solution	Complement dilution	Amount		Addition of Hemolytic amboceptor (1:2500)	Addition of 2% erythrocyte suspension.
O-Antigen of type 1 of L.monocytogenes Dilution 1:25	0.25 ml. per tube	0.25 ml. per tube	1:20 1:22 1:25 1:27 1:30 1:32 1:35 1:37 1:40 1:42 etc.	0.5 ml. per tube	Incubated 1 hour at 37°C. in water bath	0.25 ml. per tube	0.25 ml. per tube

Evaluation: Listeria antigens have the property of being able to use considerable complement. Research has shown that the quantity of complement necessary for complete hemolysis as determined in preliminary studies, is not always sufficient in primary studies in the presence of serum. Therefore for the establishment of the concentration needed for primary research a slight excess of complement is used.

If one performs the primary research with serum according to the same technique as in the preliminary research, it is important, by way of example, to use a complement dilution of 1:28 if total hemolysis in the preliminary research occurred at a complement concentration of 1:44.

This corresponds to about 1.25 complement units (for definition see below). In case of cold fixation (12-16 hours at 4°C.) one must just about double the quantity of complement (corresponding to 2 units of complement if the quantity of complement necessary to cause complete hemolysis in the presence of antigen is designated as a single unit).

The results in immune sera are absolutely clear. Complete inhibition of hemolysis shows no "after solution"; after many hours of being kept in a refrigerator the erythrocytes are precipitated and the positive tubes are easy to perceive by the overlying colorless or pale pink appearing liquid. A typical example is shown as follows:

(Table 17.)

Table 17. Performance of the complement fixation test for listeriosis on immune sera of type I in the presence of antigen of type I

Veronal buffer solution	Serum type I 0.25 ml. dilution in veronal buffer solution	Complement dilution 1:38	Antigens type I diluted 1:25		Addition of Ambo-ceptor 1:2500	Addition of 2% erythrocyte suspension.	
-	1:10	0.5 ml. per tube	0.25 ml. per tube	1 hour in water bath at 37°C. incubated	0.25 ml. per tube	0.25 ml. per tube	incubated 15 min. at 37°C. in a water bath
-	1:20						
-	1:40						
-	1:80						
-	1:160						
-	1:320						
-	1:640						
-	1:1280						
0.25 ml.	-	0.5 ml.	0.25 ml.				
0.25 ml.	0.25 ml. (1:5 dilution)	0.5 ml.	-				
1.0 ml.	-	-	-				
0.5 ml.	-	0.5 ml.					

Table 18. Results of the complement fixation test in the presence of Listeria and Enterococcus antigens.

Antigen	Technique	Reactions in dilutions of immune serums				Without serum
		L. monocytogenes Type 1 and 2 in dilutions from 1:10-1:280	L. monocytogenes Type 3 in dilutions from 1:10-1:280	L. monocytogenes Type 4 in dilutions from 1:10-1:280	Enterococcus Strain L/49[2] in dilutions from 1:10-1:280	
L. monocytogenes, Type 1 and 2	(242c)	4444420[1]	32221000	43200000	44321000	0
	(146a)	4444300	33200000	00000000	00000000	0
L. monocytogenes, Type 3	(242c)	4443200	4444210	32100000	44220000	0
	(146a)	44000000	4443000	20000000	00000000	
L. monocytogenes, Type 4	(242c)	31000000	42000000	4443100	00000000	0
	(146a)	42000000	42000000	444210	00000000	0
Enterococcus Strain L/49	(242c)	42000000	30000000	40000000	4444422	0
	(146a)	44210000	40000000	41000000	444400	0
Without Antigen	(242c)	0	0	0	0	0
	(146a)					

[1] Numbers as degrees of hemolysis inhibition occurring (4 equals 100%).

[2] Homologous agglutination titer 1:5120, L. monocytogenes type 1-O-titer 1:640.

Continuation of Table 18

Antigen	Technique	Reactions in dilutions of serums from patients: No. C 475 in dilutions of 1:5-1:160 Doubtful Listeria meningitis	No. C 503 in dilutions of 1:5-1:160 Post-abortion conditions	No. C 607 in dilutions of 1:5-1:160 Post-abortion conditions	No. C 227 in dilutions of 1:5-1:160 Post-abortion conditions	No. C 1063 in dilutions of 1:5-1:160 Mother of a newborn that died from a L. monocytogenes type 4 meningitis
L.monocytogenes	(242c)	440000	444300	444000	443000	
Type 1	(146a)			420000		440000
L.monocytogenes Type 3	(242c)	444400	421000	440000	400000	
	(146a)			400000		440000
L.monocytogenes	(242c)	000000	210000	000000	000000	
Type 4	(146a)			000000		444200
Enterococcus L/49	(242c)	000000	000000	000000	430000	
	(146a)			000000		000000
Without Antigen	(242c) (146a)	0	0	0	0	0

The examination of inactivated sera from patients took place in the same fashion, only the results stem from a lower serum dilution (for example, 1:5).

The quantity of serum used is understood in this case to be 0.25 ml. Through the addition of antigen and complement the serum is indeed still further thinned; this, however, does not have to be taken into consideration in the calculation of the end dilution.

To find the sera that react positively to the complement fixation test a single dilution is first tested against all antigens including the enterococcal control antigens. In case of positive results of the test the serum is then titered out in a followup test. For this a micro-test on an object glass has proved good, whereupon instead of 0.25 ml. sometimes one drop from graduated capillary tubes is dropped at the same angle of inclination and correspondingly mixed in. The incubation times are the same, the ingredients are stored in a damp chamber at 37°C. The ingredients used in primary research are to be seen in table 19.

Evaluation of positive results: The greater number of positive agglutination reactions are in contrast to the rarity of definitely positive reactions in the complement fixation test, if a mixed material of normal sera and patient sera is tested.

Table 19. Complement fixation test in listeriosis.
Primary research with serum from patients.

Patient's serum, dilution 1:5	Antigen	Veronal buffer	Complement dilution corresponding to preliminary test		Addition of Amboceptor 1:2500	Addition of 2% Erythrocytes suspension
	L. monocytogenes	--				
0.25 ml.	0.25 ml. Type 1/2	--	0.5 ml.	Incubated	0.25 ml.	0.25 ml.
0.25 ml.	0.25 ml. Type 3	--	0.5 ml.	1 hr. at	0.25 ml.	0.25 ml.
0.25 ml.	0.25 ml. Type 4	--	0.5 ml.	37°C.	0.25 ml.	0.25 ml.
0.25 ml.	0.25 ml. Enterococcus	--	0.5 ml.		0.25 ml.	0.25 ml.
0.25 ml.	--	0.25 ml.	0.5 ml.[1)]		0.25 ml.	0.25 ml.

1) Highest dilution that was used in the test.

Besides, the following controls were carried out with:

1. a) positive serum with veronal buffer
   b) positive serum with homologous antigen
   c) negative serum with veronal buffer
   d) negative serum with antigen
   these ingredients in all four antigens.

2. veronal buffer with antigen
   this in all four antigens.

3. a) veronal buffer, complement
   b) veronal buffer no complement
   } routine control

4. test serum, veronal buffer, no complement.

Doubtful or only weakly positive reactions in low serum dilutions are often found indeed (for example 1:5) with the technique described; however, they require no special attention. On the contrary strongly positive reactions (4 plus and 3 plus reactions) have been observed to

date almost exclusively in confirmed cases of listeriosis or apparent cases of listeriosis. The titers lie considerably lower than the homologous agglutination titer and only exceptionally reaches the values above 1:80. Titers over 1:10 are in our opinion significant, and were also thus considered by Patocka who saw 3 plus reactions up to 1:256. Patocka found moreover in studies of sera of healthy women in childbed, etc., with relative frequency a thrice positive complement fixation test in serum dilutions of 1:4 to 1:32 so that the limits of specificity seem to be 1:32.

Unfortunately the details of his method are not known; therefore a direct comparison is not possible to date.

We believe according to our own investigations, however that with our technique a strongly positive reaction is already present at a titer of 1:5, and 3 plus titers of 1:10 upwards in the presence of type I and type 4 antigens indicate the presence of a Listeria infection, while these kind of titers are to be considered the upper limits in the case of a bacteriologically confirmed infection.

The overlapping reactions between Enterococci and Listeria antigens, require particularly careful evaluation.

The results yielded with immune rabbit serum may not without further ado be carried over into human sera results. In practice we have to date despite numerous positive findings only relatively seldom

observed a concomitant reaction with enterococcic antigen. In the same titer the result is only to be evaluated after subsequent saturation.

While the evaluation of the results of the use of type 1 and type 4 antigens offers no difficulty, it is important to evaluate positive results with type 3 antigen only in connection with the H-agglutination titers, since not seldom nonspecific reactions appear, the source of which is not yet explained.

The reaction results are naturally considerably influenced by the technique. If one uses a slight excess of complement in primary research (242c) the test is, on the whole, more sensitive. Cross reactions then appear stronger and more often than with the use of a higher dose of complement (2 units) as is used today in the complement fixation test for the presence of viral antigens (146a). In table 18 the results of similar investigations with the two test methods are compared in both animal and human sera. They show an increased specificity with an increased quantity of complement which however is many times paid for by a loss of titer.

Oezgen brought up for discussion whether during the course of a listeriosis due to virus the formation of complement fixing Listeria antibodies would be stimulated. In similar research material sera with Paul-Bunnell titers of over 1:64 regularly gave one plus and

2 plus reactions in dilutions of 1:5 in the presence of type I antigen. At least as frequent were however Listeria complement fixation reactions negative even in the case of high Paul-Bunnell titers. Patocka likewise has observed positive Listeria complement fixation in cases of monocytic angina follicularis.

So far as the present investigations permit a conclusion, there are no serologic cross reactions between Wassermann positive and Listeria positive sera, although both antigens contain abundant lipoid. On the other hand it should be pointed out that in human cases of listeriosis a positive Wassermann reaction has been observed at the beginning of the disease (31,151) which later vanished. Our own investigations (242j) on ten sera in which the Wassermann reaction was strongly positive, showed no deviation of complement in the presence of Listeria-antigens, and vice versa the Wassermann reaction was indisputably negative in ten sera with high Listeria titers.

On the contrary, there are not seldom positive Meinicke reactions in listeriosis sera, obviously as a result of the generally present liver damage.

As already stated, the Listeria titers in the complement fixation test are considerably lower than in the agglutination test. The titer value is decisively influenced by the time at which the investigation is made. Namely, it has been shown that the complement fixing antibodies

are only detectable for a short time, and diminish relatively swiftly or may even disappear after clinical healing. Many times titers of 1:40 or 1:80 become negative in 2 or 3 weeks, indeed sometimes within 10 days.

It is not seldom observed that the agglutination titer even after the return to negative or the diminution of the positive complement fixation test values can still rise. Individual instances show relatively high complement fixing titers (1:20) with relatively low agglutination titers with homologous antigen (1:80 to 1:160) others show high agglutination titers (1:1280 and over) in case of indisputably negative complement fixation reactions.

The findings confirm the hypothesis that the complement fixing and agglutinizing antibodies are not unique, and therefore also have a different significance. Agglutinins are more easily formed and remain detectable much longer. Complement fixing antibodies appear in case of listeriosis many times only as transitory phenomena.

c) Other procedures-- On the basis of research results with sera from animals Drew considered the possibility that soluble Listeria substances appear in the spinal fluid of listeriosis patients that should be detectable with precipitating sera. This has only infrequently been proved to date. A few investigations of our own ran negatively.

Conversely it was to be deduced that, analogous to the appearance

of agglutinins and complement fixing antibodies, precipitins should also appear in the sera of patients, that could be detected with the concomitant precipitinogen. Potel has made abundant use of the precipitation test, and has obtained in a relatively high percentage of sera with high agglutinin content a positive reaction with a soluble Listeria precipitinogen.

The manufacture of the precipitinogen according to Potel: The growth of L. monocytogenes on meat broth agar is suspended in 5 ml. of bouillon extract, and incubated 3 days further at 37°C. The undiluted Seitz filtrate of this bouillon serves as precipitinogen.

On the contrary we only seldom succeeded in getting a definite precipitation from Fuller antigen or trichloracetic acid extracts of Listeria in the presence of human sera which yielded an agglutination titer of 1:160 to 1:1280 and with the complement fixation test reacted 3 plus up to 1:80.-- With artificially immunized animals it may be shown that precipitins against the bacterial polysaccharide from all antibodies appear last and in the smallest amounts.

Later investigations carried out jointly with Potel with human sera with the use of different lysates and extracts speak particularly against the use of precipitin tests in the practical diagnosis of listeriosis.

Perhaps we may however investigate with sensitized blood cells for a refinement (of the technique) and yet proceed thereby to valuable results.

Intracutaneous tests with Listeria antigen have been carried out only infrequently to date and led for example in the studies of Harvier, cited above, to dubious results. The value of this method is being tested at present, so that it is here still too early to draw a conclusion about it.

Conclusion.

The diagnosis of listeriosis is possible only by bacteriologic and--with certain limitations--by serologic methods.

As material for investigation for bacteriologic detection during life, blood, spinal fluid, bone marrow, (sternal), amniotic fluid, meconium, throat, and other mucosal scrapings (swabs), first come under consideration. After death the search for the microorganism is successful, in addition in liver, spleen, lymph nodes, cerebrum, cerebellum, medulla, and other organs as well.

The culture of the microorganism occasionally presents difficulties that may be overcome partly by preliminary culture in highly valuable media, partly by a weeklong period of enriched growth at 4°C. In the isolation of Listeria from material containing the microorganism in the usual way, tellurite media and the use of the plate microscope give good service. Morphologic, cultural, biochemical, and serologic similarities to other kinds of bacteria necessitate an exact differential diagnosis. The possibilities of error are

obviously quite large. Confusion with similar bacteria often occurs. This applies particularly to Enterococci and Corynebacteria.

During the course of listeriosis men and animals form humoral antibodies, that are detectable by means of the Widal agglutination test. Listeria-agglutinins are found however also in increasing frequency in sera of healthy persons or animals without any classic history of listeriosis. The question as to the specificity of Listeria agglutinins is not yet sufficiently clarified.

Heteroagglutinations in Enterococcus sera exist. Therefore the Widal reaction is useful in Listeria diagnosis only in case of critical evaluation. Titers over 1:320 and titer rises of at least two stages have a certain value as proof. Lately successful improvement of the serodiagnosis has been accomplished with a complement fixation test that is carried out with the different antigens. In the majority of the patients complement fixing antibodies appear during the disease and in the period of convalescence.

The serologic tests are according to their value as proof less than the bacteriologic investigations, but indicate, however, useful conclusions in cases in which the culture of the microorganism is not, or at the time of the investigation is no longer, successful.

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